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WATER QUALITY REQUIREMENTS OF AQUATIC INSECTS

Arden R. Gaufin

Utah University

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To maintain a well-rounded diversified population of cold water aquatic insects, maximum temperatures, minimum dissolved oxygen levels, and the pH range should not exceed the requirements of cold water fishes, such as trout and salmon. While some aquatic insects can tolerate dissolved oxygen levels as low as 1.6 mg/l for short periods, concentrations of 6.0 mg/l are required for long-term survival. Temperatures during the winter months must be maintained at normal seasonal levels to prevent premature emergence. Temperatures above 65° F during the summer months are considered the maximum for maintaining many species of stoneflies, mayflies, and caddis flies. A pH range of 6.0 - 8.5 should protect most cold water lotic insects. (EPA)

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WATER QUALITY REQUIREMENTS OF AQUATIC INSECTS

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ABSTRACT

The larvae of twenty species of aquatic insects (Diptera, Ephemeroptera, Plecoptera, and Trichoptera) and the scud (Amphipoda) were exposed to high water temperatures, low dissolved oxygen concentrations, and low pH to determine their tolerance of these three environmental factors. The temperature at which 50% of the specimens died after 96 hours exposure ranged from 11.7° C for the mayfly, Cinygmula par Eaton, to 32.6° C for the snipe fly, Atherix variegata Walker. The mayfly, Ephemerella doddsi Needham, was most sensitive to low dissolved oxygen levels with a 96-hour TLm of 5.2 mg/l. Acroneuria pacifica Banks, a stonefly, was the most resistant with a TLm^{96} of 1.6 mg/l. Median tolerance levels for pH ranged from pH 2.7 for the caddis fly, Limnephilus ornatus Banks, to 7.2 for the scud, Gammarus limnaeus Smith. Longer term bioassays clearly indicated increased sensitivity and mortality of the test specimens with increased length of exposure to each of these factors.

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CONCLUSIONS

1. Acroneuria pacifica, a stonefly, was the most resistant form tested to low oxygen concentrations with a TLm^{96} of 1.6 mg/l; Ephemerella doddsi, a mayfly, was the most sensitive with a TLm^{96} of 5.2 mg/l.

2. The group most tolerant of low dissolved oxygen levels was the Trichoptera (2.86 mg/l).

3. Water flow is very important in determining dissolved oxygen limits. The mean TLm^{96} for 10 species of aquatic insects tested at 500 cc/min flow was 3.64 mg/l; the mean for 10 species at 1000 cc/min was 2.55 mg/l.

4. Increased sensitivity and mortality of test specimens occurred with increased length of exposure to low oxygen levels. Whereas, 50% of the specimens of Acroneuria pacifica survived an oxygen concentration of 1.6 mg/l for 4 days, the minimal oxygen level for 50% survival at 111 days was 5.8 mg/l.

5. Atherix variegata, a Dipteran, was the most tolerant of high water temperatures with a TLm^{96} of 32.6° C; Cinygmula par, was the most sensitive with a TLm^{96} of 11.7° C.

6. Acclimation to colder temperatures in nature results in increased sensitivity to exposure to elevated temperatures. For example, specimens of the stonefly, Isogenus aestivalis, from Utah were much more tolerant than Montana specimens with a TLm^{96} of 24.2° C in comparison to a TLm^{96} of 16.1° C for Montana specimens.

7. Increased mortality of test specimens occurred with increased length of exposure to high temperatures. The TLm^{96} for specimens of the stonefly, Pteronarcella badia, from Montana was 24.4° C. In comparison, 50% of the specimens succumbed to a temperature of 18.1° C in 24 days.

8. Exposure to sublethal temperatures increases growth rate and emergence. Pteronarcella badia, a stonefly, which normally emerges in mid June, emerged early in February after exposure to a temperature of 17° C for 29 days.

9. Limnephilus ornatus, a caddis fly, was the most tolerant of low pH levels with a TLm^{96} of 2.7; Gammarus limnaeus, the scud, was the most sensitive with a TLm^{96} of 7.2.

10. The results of both short and long-term bioassays indicated that mayflies are most sensitive to low pH levels with stoneflies being moderately sensitive and caddis flies least sensitive.

11. Exposure for short periods to pH levels well below those normally found in nature may not be harmful. Longer exposure, however, may have decidedly detrimental effects on molting, growth, and emergence.

RECOMMENDATIONS

1. To maintain a well-rounded diversified population of cold water aquatic insects, maximum temperatures, minimum dissolved oxygen levels, and the pH range should not exceed the requirements of cold water fishes, such as trout and salmon. While some aquatic insects can tolerate dissolved oxygen levels as low as 1.6 mg/l for short periods, concentrations of 6.0 mg/l are required for long-term survival. Temperatures during the winter months must be maintained at normal seasonal levels to prevent premature emergence. Temperatures above 65° F during the summer months are considered the maximum for maintaining many species of stoneflies, mayflies, and caddis flies. A pH range of 6.0 - 8.5 should protect most cold water lotic insects.

2. Since aquatic insects are much more sensitive during molting and emergence, further research should be undertaken to determine the effects of these and other environmental factors on the most sensitive stage of the most common species of aquatic invertebrates.

3. Inasmuch as there is considerable variation in the environmental requirements of different species of aquatic insects, further research is needed on a country-wide basis to set criteria for the protection of both cold water and warm water species in various types of habitat.

INTRODUCTION

Industrial and population expansion in many areas has resulted in badly polluting our streams. Among the results of pollution is the reduction or even the depletion of dissolved oxygen in the water. The amount of oxygen dissolved in water was cited by Reish and Richards (1966) as perhaps the single most important environmental factor for the survival, growth, and reproduction of aquatic animals. The oxygen content of the water during nymphal growth was considered by Per Brinck (1949) as one of the most important factors in the distribution of stoneflies. In his studies in south Swedish waters he showed that sections of streams with a low oxygen content (below 40% saturation) had an insignificant or no stonefly fauna.

Water temperatures have a profound and diverse effect on aquatic life. Uncontrolled high water temperatures may have a directly lethal effect and serve as a barrier to movements of river-migrant fishes. Continuously high water temperatures may prevent production of desirable game fishes and other aquatic species and result in their eventual elimination. High water temperatures may cause extensive ecological changes in rivers and lakes and drastically alter the biota. Limited quantities of warm water, however, may produce desirable changes in selected localized situations.

In coal mining regions of the United States water pollution by acid mine drainage constitutes a problem of major importance. Pollution by acids may be sufficient to not only make the water of a stream unfavorable for the growth and development of fish and aquatic invertebrates but there may also be a directly lethal effect.

Those discharging wastes into our waters need to know the requirements of aquatic life in order to ascertain the amount of waste which can be introduced into our streams without jeopardizing the conditions necessary to maintain aquatic life. Water quality criteria for the protection of aquatic life must be established, but there is a lack of agreement among workers as to just what these criteria should be or how they shoud be applied. Any criteria that are established must be based on a knowledge of habitat requirements for those forms inhabiting the particular body of water under consideration. Such criteria must encompass all environmental factors necessary for the survival, growth, reproduction, and well being of the aquatic organisms. Each species should be evaluated at the various stages in its life history if such criteria are to serve their purpose.

Biological examinations have been used for many years to assess the degree of pollution of our lakes and streams. Immature aquatic insects have been used extensively as biological indicators because

of their sensitivity to changes in their environment, the length of their life cycles, and their lack of mobility in comparison to fish. Over the years fairly extensive lists of aquatic insects indicative of various degrees of water pollution have been published by such workers as Kolkwitz and Marsson (1909), Fair and Whipple (1948), Liebmann (1951), Gaufin and Tarzwell (1953, 1956), Hynes (1960), and others. Disagreement over the exact status of many of these organisms exists because of differences in chemical and physical conditions at the time of sampling and insufficient knowledge concerning the environmental requirements of the organisms collected.

Our knowledge of the requirements of individual species of aquatic insects is extremely limited. For many of our North American species life cycles are unknown, immature stages are undescribed, and the total span of emergence periods unrecorded. Only a few species have been the subject of detailed study.

Laboratory experiments have been conducted at the University of Utah for the last ten years in order to better isolate, understand, and interpret some of the environmental factors which have an important effect on the behavior and physiological reactions of stoneflies. The specific objectives of this work have been to determine the effects of low dissolved oxygen concentrations at various temperatures and water flow on the gross activity of stoneflies, to determine the minimum dissolved oxygen concentrations at which exposure for a prolonged period of time can be endured without lethal effects, to determine metabolic levels of various species of stoneflies, and to determine the food habits of as many species as possible.

Ecological studies of the environmental requirements of various species of aquatic insects in the Intermountain Region have been conducted at the University of Utah since 1946. Considerable data has been collected as to the effects of pollution on the biota present in a number of streams. Considerable data as to the biota present, productivity, and chemical-physical characteristics of a number of streams, such as the Provo, Weber, and Jordan Rivers in Utah; Colorado River in Colorado, and Bitterroot River in Montana, have been accumulated.

This report summarizes three years of research which focused upon the effects of low dissolved oxygen levels, high temperatures, and an acid environment on aquatic insects. The objectives of the research were to determine lethal and sublethal levels, and acute and long-term effects of these factors on the survival, growth, reproduction, and behavior of 20 species of aquatic insects and the scud, Gammarus limnaeus. Gammarus limnaeus in this report is considered as a subspecies of Gammarus lacustris.

STUDIES ON THE TOLERANCE OF AQUATIC INSECTS

TO LOW OXYGEN CONCENTRATIONS

Introduction

Oxygen is a basic need of aquatic insects, yet information concerning their exact oxygen requirements is known for but a very few species. Gaufin and Tarzwell (1956) pointed out that if the oxygen requirements of different species of aquatic insects were better known, it should be possible to estimate in retrospect, with considerable accuracy, what oxygen levels have existed in a given aquatic environment during the life history of the organisms. Thus aquatic insects could be used as an excellent index of water quality.

The literature is extensive on oxygen consumption by various animals, yet such values are meaningful only for the particular conditions of measurement. The conditions under which such measurements were made are important because the rate of oxygen consumption is influenced by several internal and external variables. The rate of oxygen consumption is influenced by activity, temperature, nutrition, body size, stage in life cycle, season, and time of day, as well as by previous oxygen experience and genetic background (Prosser and Brown, 1961). The highest respiratory rates usually occur in the small, very active forms; whereas, the lowest occur in the large relatively sedentary forms.

Wigglesworth (1950) and Edwards (1946) summarized much of the work that has been done on respiration rates of insects. The majority of the publications on immature aquatic insects has been on European species. Extensive work on individual, immature, aquatic insects was done by Balke (1957) on European species of the orders Neuroptera, Odonata, Plecoptera, and Trichoptera. The difficulty in selecting a suitable and adequate method for the measurement of the respiratory rate in a particular species of aquatic insect was evaluated by Kamler in 1969. An analysis of the various factors which influence the oxygen requirements and respiratory rates of benthic invertebrates is presented in "The Ecology of Running Waters" by Hynes (1970). The oxygen consumption of ten of the most common species of stoneflies of the western United States and the factors which modify their metabolic rate are discussed by Knight and Gaufin (1966). The oxygen requirements of immature aquatic insects in relationship to their classification as index organisms are thoroughly evaluated by Olson and Rueger (1968). Their statistical analyses of oxygen consumption rates by twelve representative

species of aquatic insects of the upper Great Lakes Region constitute very valuable data for establishing water quality criteria for the protection of aquatic life.

The principal objectives of the studies presented in this report were to determine the oxygen requirements of representative species of aquatic insects of the Intermountain Region and to determine their relative sensitivity to low oxygen concentrations. Oxygen levels necessary for survival and the long-term effects of low oxygen concentrations on molting, growth rates, time of emergence and behavior patterns were investigated.

This report summarizes the results of acute, short-term 96-hour tests (TLm^{96}) used in screening 20 species of aquatic insects to determine their relative sensitivity to low oxygen concentrations. In addition, the longer term effects of low oxygen levels on the survival, molting, growth, time of emergence, and behavior patterns of 21 species are considered. The 96-hour TLm (Standard Methods, 1965) was used as a measure of survival in the tests. This report encompasses work conducted at the University of Montana Biological Station during 1968-70 and at the University of Utah in 1966, 1970-71.

Materials and Methods

The organisms used in the tests were all insects except for one species of Amphipoda. All organisms were collected from streams and ponds in northwestern Montana and in northern Utah. Care was taken to ensure that the organisms for a test were all collected from the same area at the same time. The specimens were kept in well oxygenated holding tanks for three days prior to testing. Only specimens of the same age group were utilized. These were generally of the oldest year class present. Test procedures were those outlined in Standard Methods (1965).

De-oxygenated water was obtained from degassing equipment as described by Mount (1965). Modifications included a cooling system and an oxygen "ladder." The ladder is constructed of single pane glass and cemented with silicone sealant. The ladder is 5-1/2 feet long, 7 inches wide and 7 inches deep. It is divided into 15 compartments each separated by a glass partition 2 inches high. The remainder of the divider is composed of fiberglass screen with a 1 mm mesh opening.

The de-oxygenated water comes from the degasser through plastic tubing, passes through the cooler and then enters one end of the ladder which is elevated above the outlet end. As the water flows over the 2-inch compartment dividers its oxygen content increases. Rates of increase are dependent upon rate of inflow and the angle of inclination of the ladder. At an inclination of 40° from the horizontal

and a flow rate of 1000 cc/min the oxygen increase per chamber is about 0.5 mg/l at 10° C.

Ten organisms were placed in each of seven test chambers and observed twice daily. Point of death was determined by lack of response when stimulated. Small rocks were placed in the test chambers to which the organisms could cling.

The flow rate was checked weekly and varied plus or minus 25 cc/min. The temperature was taken daily with a pocket thermometer. A variation of plus or minus 0.5° C occurred. Oxygen concentration was taken daily using the modified Winkler method, utilizing a 50 ml sample. Variations of plus or minus 0.2 mg/l occurred.

Water used in the tests at the Biological Station was unchlorinated well water with the following chemical composition: pH 7.8; total hardness, 135 mg/l; temperature, 6.4° C; turbidity, 0-5 J.T.U.; carbon dioxide, 1-2 mg/l.

Results

Nineteen species of aquatic insects and one species of Amphipoda were studied to determine their 96-hour median tolerance limit (TLm). Eight species of Plecoptera were tested. The mean TLm for this group was 3.04 mg/l of oxygen. Acroneuria pacifica Banks had the lowest Tlm, 1.6 mg/l at a flow rate of 1000 cc/min (Table 1). The highest TLm was obtained with Pteronarcys californica Newport (3.9 mg/l) at a rate of 500 cc/min. The TLm for this species decreased to 3.2 mg/l at a flow of 1000 cc/min. All of the specimens of Arcynopteryx parallela Frison survived at oxygen concentrations of 2-5.00 mg/l at a flow of 1000 cc/min. All of the test species were stream forms.

Four species of mayflies (Ephemeroptera) were examined. Two species were lotic forms, Hexagenia limbata Guerin and Callibaetis montanus (Eaton). Their TLm's were 1.8 mg/l and 4.4 mg/l respectively. The lentic forms tested were Ephemerella doddsi Needham and Ephemerella grandis Eaton, with D.O. values of 5.2 mg/l and 3.0 mg/l respectively. The mean for the group was 3.6 mg/l.

Table 1

Test Organisms, TLm in mg/l, Per Cent Saturation and Water Flow in cc/min

Organisms	TLm	Saturation	Flow
PLECOPTERA			
Acroneuria pacifica Banks	1.6	14	1000
Arcynopteryx aurea Smith	3.3	29	1000
Arcynopteryx parallela Frison	100% Survival	2-5 mg/l	1000
Diura knowltoni (Frison)	3.6	32	500
Nemoura cinctipes Banks	3.3	29	1000
Pteronarcys californica Newport	3.9	34	500
" " "	3.2	28	1000
Pteronarcella badia (Hagen)	2.4	21	1000
EPHEMEROPTERA			
Callibaetis montanus Eaton	4.4	38	500
Ephemerella doddsi Needham	5.2	46	500
Ephemerella grandis Eaton	3.0	27	1000
Hexagenia limbata Guerin	1.8	15	1000
TRICHOPTERA			
Brachycentrus occidentalis Banks	90% Survival	2-4 mg/l	500
Drusinus sp.	1.8	15	1000
Hydropsyche sp.	3.6	32	500
Lepidostoma sp.	80% Survival	3-4 mg/l	1000
Limnephilus ornatus Banks	3.4	30	500
Neophylax sp.	3.8	33	500
Neothremma alicia Banks	1.7	14	500
DIPTERA			
Simulium vittatum Zetterstadt	3.2	28	500
AMPHIPODA			
Gammarus limnaeus Smith	80% Survival	3 mg/l	500

Seven species of Trichoptera were tested and all were from lentic environments. Several of these organisms could not be identified to the species level. Ninety percent of the specimens of Brachycentrus occidentalis Banks survived at oxygen concentrations of 2-4 mg/l and a flow rate of 500 cc/min. Neothremma alicia Banks, a small species (5 mm), had the lowest TLm of 1.7 mg/l. Neophylax sp. had the highest TLm of 3.8 mg/l. The mean for the entire group was 2.86 mg/l.

One Dipteran was tested (Simulium vittatum Zetterstadt) and had a TLm of 3.2 mg/l. One Amphipoda was examined (Gammarus limnaeus Smith) with a survival of 80% at 3 mg/l of oxygen and a flow rate of 500 mg/l.

The mean TLm for all organisms tested was 3.1 mg/l. The mean for all organisms tested at a flow of 1000 cc/min was 2.55 mg/l and 3.64 mg/l at a flow of 500 cc/min. The lowest TLm recorded was 1.6 mg/l for Acroneuria pacifica, or 14% oxygen saturation. The highest TLm was 5.2 mg/l for Ephemerella doddsi, or 46% oxygen saturation.

Discussion

Of the organisms tested the group most tolerant to low dissolved oxygen (D.O.) values was the Trichoptera (2.86 mg/l). All of the Trichoptera tested, except Hydropsyche, were cased forms and all came from lentic environments. All the organisms except Drusinus sp. were tested at a flow rate of 500 cc/min. Higher flow rates would probably reduce the Tlm of many of the forms.

Acroneuria pacifica, a predacious stonefly, was the most resistant form tested with a TLm of 1.6 mg/l (14% saturation). The largest organism tested, Pteronarcys californica, showed a decrease in TLm as the flow rate increased (3.9 mg/l to 3.2 mg/l).

The mayfly, Ephemerella doddsi, had the highest TLm of 5.2 mg/l (46% saturation) at 500 cc/min. This species is found in fast streams attached to rocks.

It has been shown by Knight and Gaufin (1963, 1964) that rate of water flow is very important in determining tolerance limits. This was again demonstrated by Pteronarcys californica as did the ranges and means for the flow rates. The TLm range for 11 species tested at 500 cc/min was 1.7 mg/l to 5.2 mg/l, with a mean of 3.64 mg/l. At 1000 cc/min the range for 10 species was 1.6 mg/l to 3.3 mg/l with a mean of 2.55 mg/l, a substantially lower value.

Behavior of the organisms during testing was of interest. All of the Plecoptera initiated "push-up" movements upon introduction to the test chambers. Most species ceased this motion after several hours but Pteronarcys californica continued these movements periodically

throughout the test. Pteronarcys californica also assumed a position half out of the water in the low oxygen chambers. Nemoura cinctipes assumed a stilted position upon death.

Number of gill beats per unit time was indicative of oxygen concentration. Gill beats in Ephemerella grandis were counted after 12 hours in the test chambers and results are given in Table 2. Each value is the mean number of beats for the ten organisms in each chamber.

Table 2

Gill beats/minute for Ephemerella grandis Eaton

Oxygen conc.	Beats	Rhythm
2.4 mg/l	176	steady
3.0 "	192	"
3.6 "	192	"
4.6 "	184	erratic
5.0 "	160	"
6.0 "	100	"

Except at the lowest D.O. concentration, the gill beat decreased as the oxygen increased. The rhythm of gill beats also became erratic as the oxygen increased.

The high TLm of the pond mayfly, Callibaetis montanus, was surprising. It had the second highest TLm of all species tested (4.4 mg/l). Another lotic species Hexagenia limbata had a low TLm of 1.8 mg/l. This could probably be explained by its acclimation to lower oxygen concentrations in its normal environment.

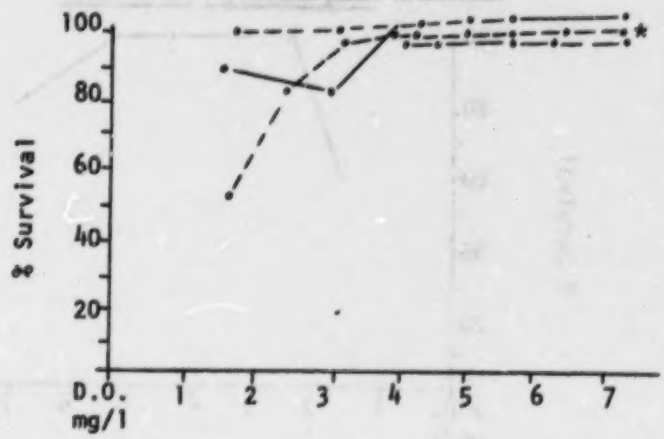
In response to the low oxygen values the Trichoptera undulated their abdomens in their cases. Simulium vittatum congregated on the chamber walls where the flow was the greatest. Gammarus limnaeus showed no behavioral response to the low oxygen values.

96-Hour TLm Results

Oxygen

1000 cc/min

Acroneuria pacifica



* Replicate tests

1000 cc/min

Arcynopteryx aurea

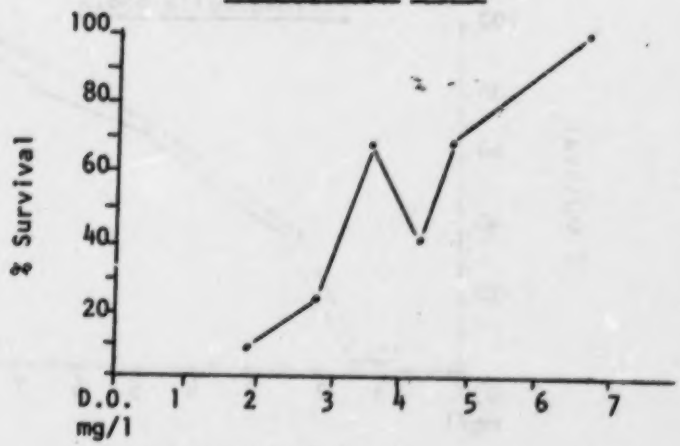


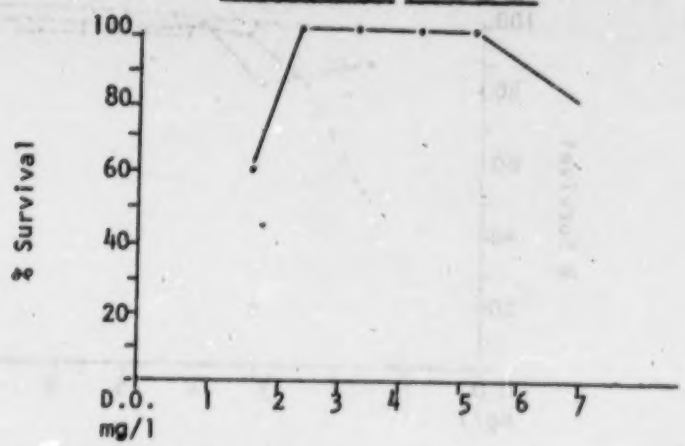
Figure 1

96-Hour TLm Results

Oxygen

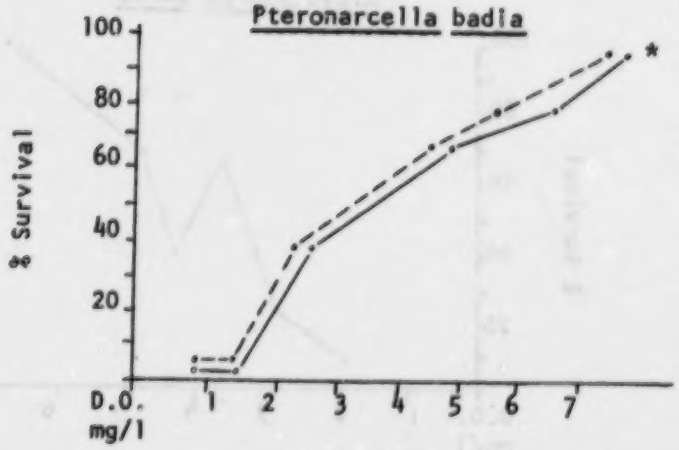
1000 cc/min

Arcynopteryx parallela



1000 cc/min

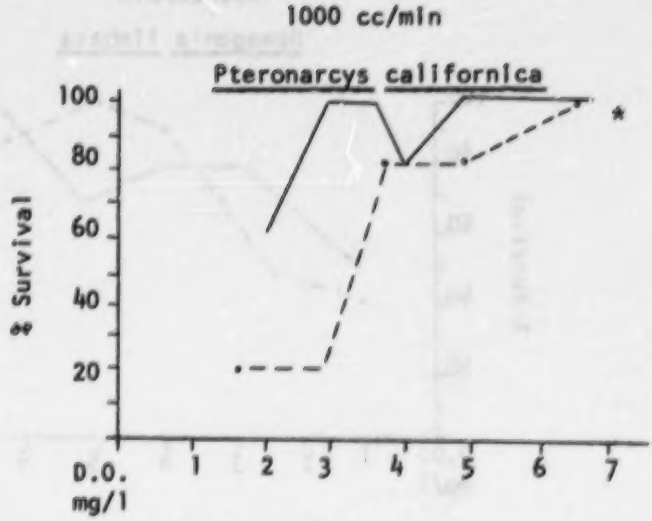
Pteronarcella badia



* Replicate tests

96-Hour TLm Results

Oxygen



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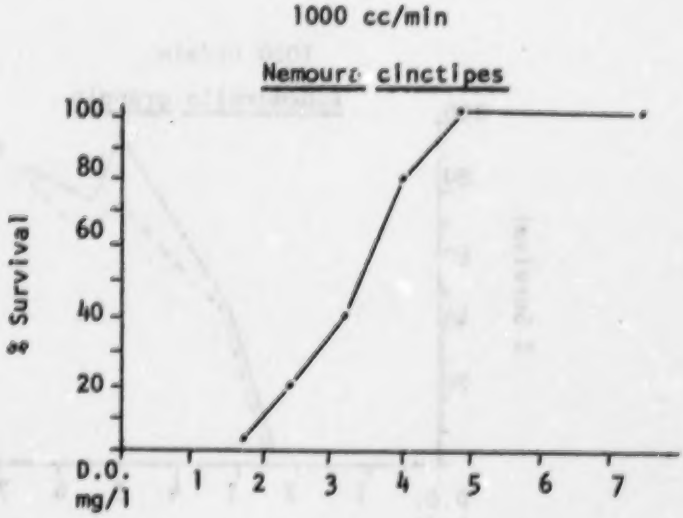
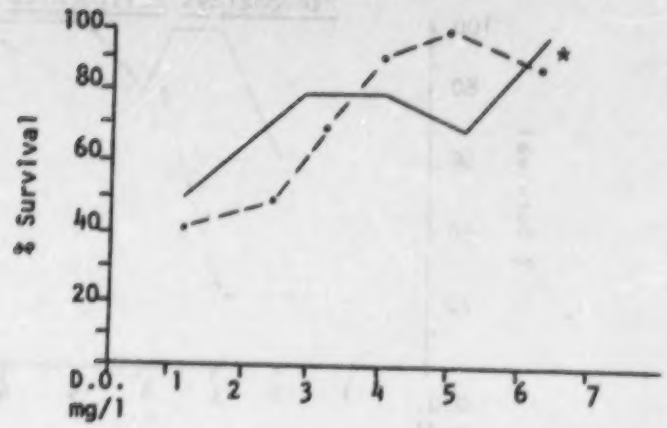


96-Hour TLm Results

Oxygen

1000 cc/min

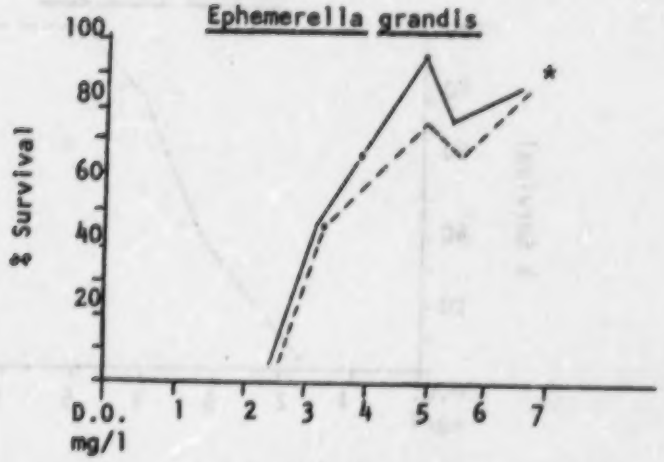
Hexagenia limbata



* Replicate tests

1000 cc/min

Ephemerella grandis



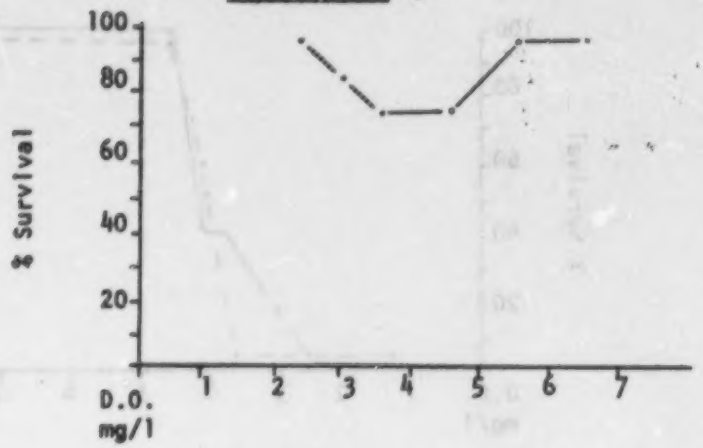
* Replicate tests

96-Hour TLm Results

Oxygen

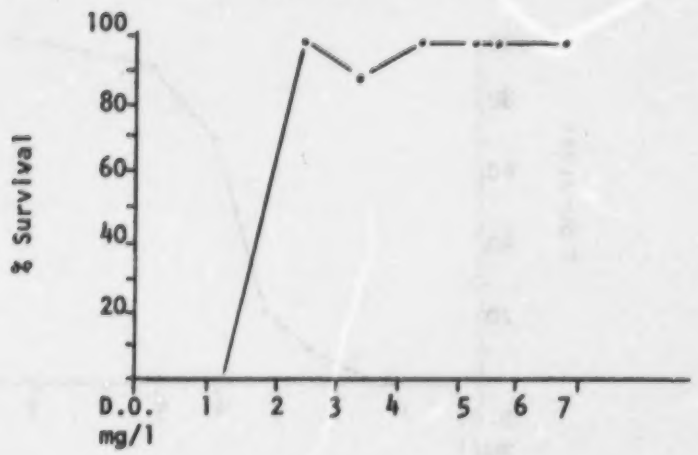
1000 cc/min

Lepidostoma sp.



1000 cc/min

Drusinus sp.

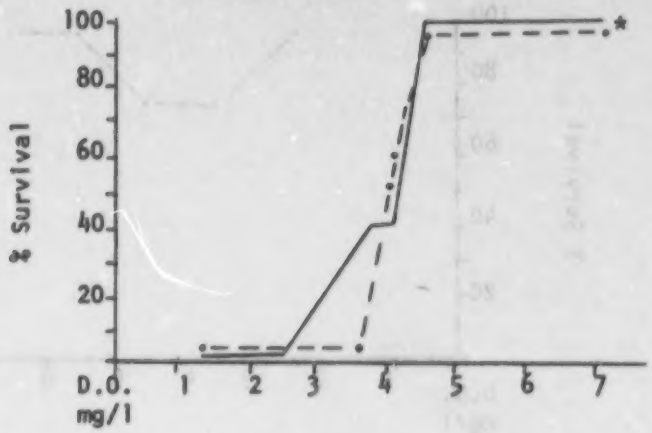


96-Hour TLm Results

Oxygen

500 cc/min

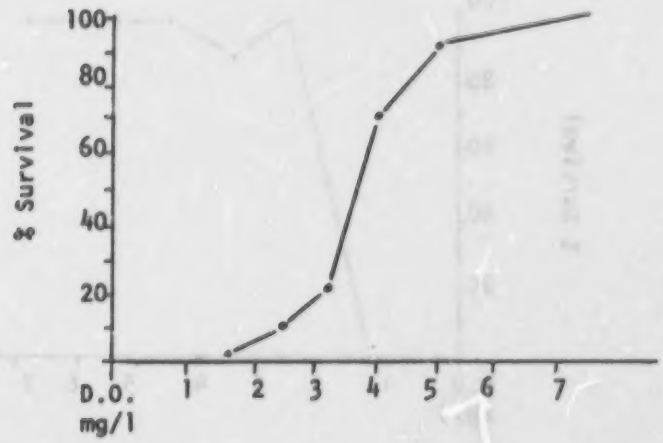
Pteronarcys californica



* Replicate tests

500 cc/min

Diura knowltoni

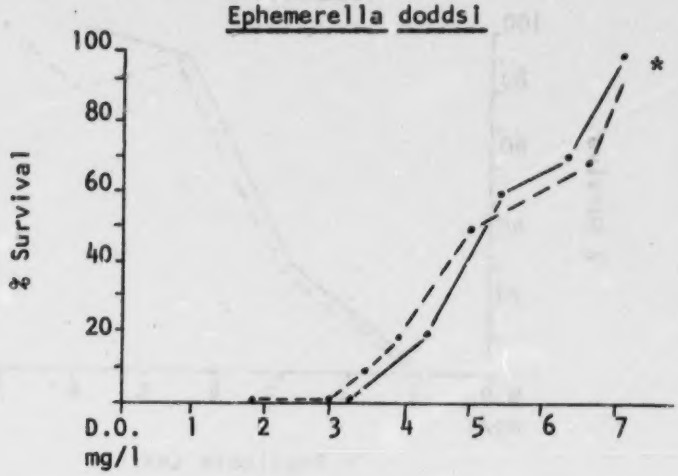


96-Hour TLm Results

Oxygen

500 cc/min

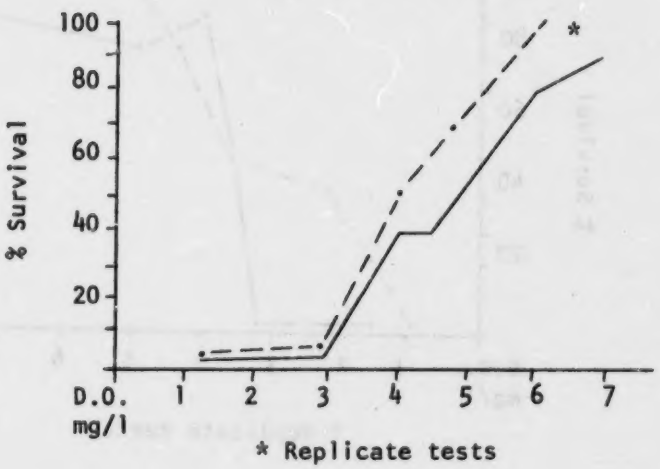
Ephemerella doddsi



* Replicate tests

500 cc/min

Callibaetis montanus



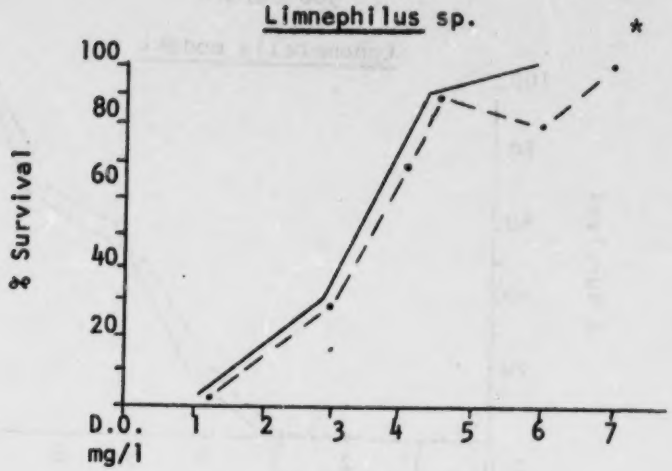
* Replicate tests

96-Hour TLm Results

Oxygen

500 cc/min

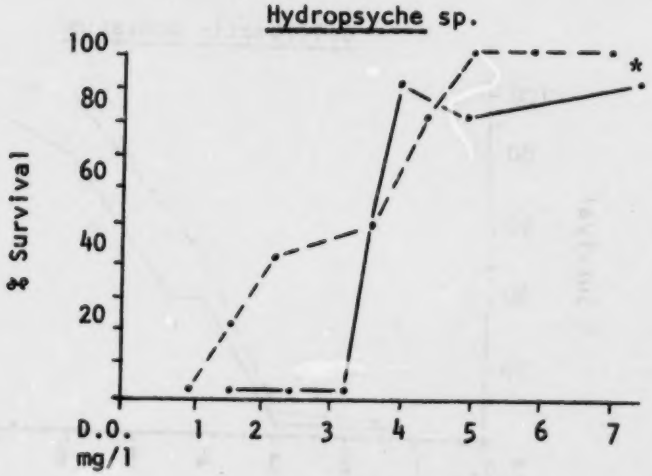
Limnephilus sp.



* Replicate tests

500 cc/min

Hydropsyche sp.



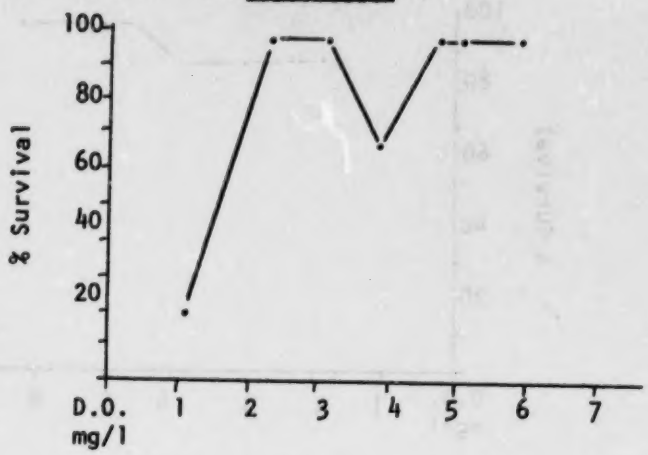
* Replicate tests

96-Hour TLm Results

Oxygen

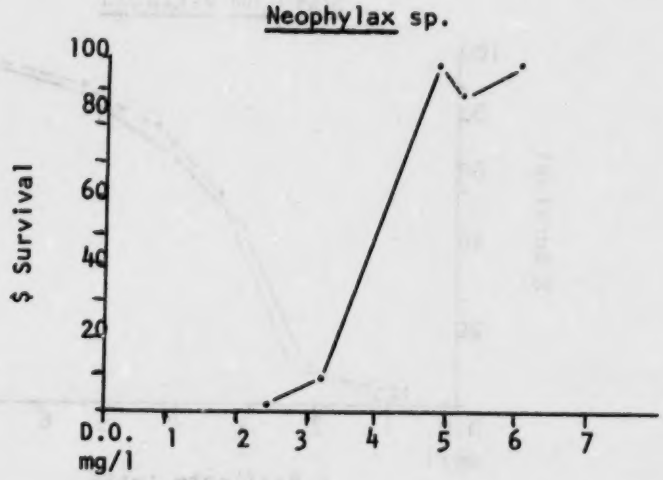
500 cc/min

Neothremma sp.



500 cc/min

Neophylax sp.

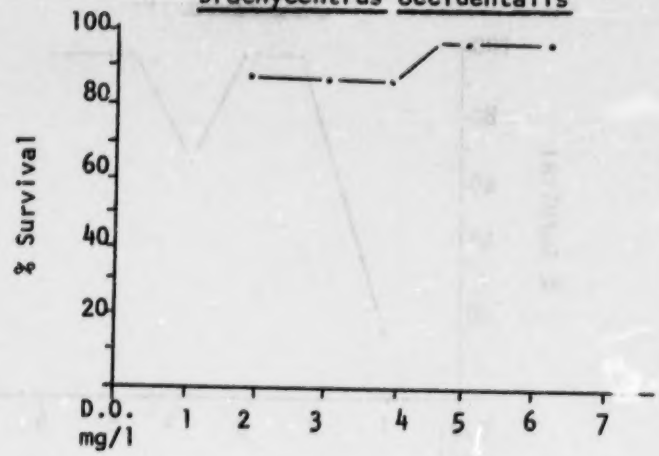


96-Hour TLm Results

Oxygen

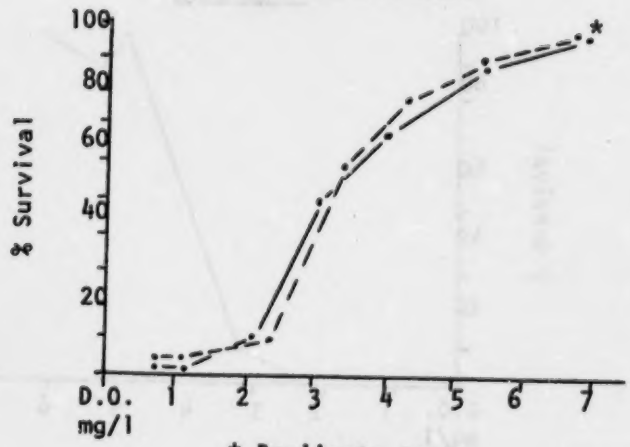
500 cc/min

Brachycentrus occidentalis



500 cc/min

Simulium vittatum



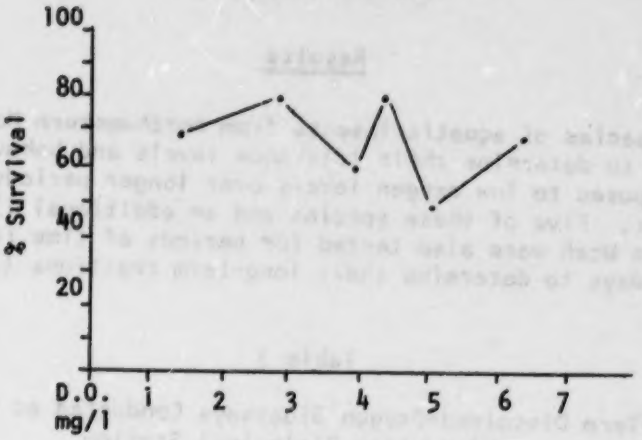
* Replicate tests

96-Hour TLm Results

Oxygen

500 cc/min

Gammarus limnaeus



LONG-TERM BIOASSAYS CONDUCTED AT THE

UNIVERSITY OF MONTANA BIOLOGICAL STATION

AND THE

UNIVERSITY OF UTAH

Results

Eight species of aquatic insects from northwestern Montana were studied to determine their tolerance levels and behavior patterns when exposed to low oxygen levels over longer periods of time than 96 hours. Five of these species and an additional 13 species from northern Utah were also tested for periods of time ranging from 4 to 104 days to determine their long-term reactions (Tables 3,4,5).

Table 3

Long-Term Dissolved-Oxygen Bioassays Conducted at University of Montana Biological Station

Species	Minimum D.O. level (mg/l)	% Survival	Survival time (days)
PLECOPTERA			
Pteronarcella badia (Hagen)	4.4	50%	69
Pteronarcys californica Newport	4.8	40%	97
Arcynopteryx aurea Smith	4.8	30%	12
Acroneuria pacifica Banks	5.8	50%	111
EPHEMEROPTERA			
Ephemerella grandis Eaton	4.6	30%	30
TRICHOPTERA			
Brachycentrus occidentalis Banks	3.2	50%	120
Hydropsyche sp.	4.8	30%	50
DIPTERA			
Atherix variegata Walker	2.4	90%	40
AMPHIPODA			
Gammarus limnaeus Smith	2.8	50%	20

Flow rate of 1000 cc/min

Table 4

Long-Term Dissolved-Oxygen Bioassays Conducted at the University of Utah (50% + Survival)

Species	Minimum D.O. level (mg/l)	% Survival	Survival time (days)
PLECOPTERA			
Acroneuria pacifica Banks	3.0	50%	24
Brachyptera nigripennis (Banks)	2.3	60%	4
Isoperla fulva Claassen	2.3	50%	13
EPHEMEROPTERA			
Ephemerella grandis Eaton	3.3	50%	18
Rhithrogena robusta Dodds	3.3	50%	7
	3.3	50%	4
TRICHOPTERA			
Brachycentrus occidentalis Banks	2.6	80%	91
Rhyacophila sp.	1.4	50%	45
Arctopsyche grandis (Banks)	3.4	50%	26
Parapsyche elsis Milne	5.2	60%	30
DIPTERA			
Atherix variegata Walker	2.4	90%	97
Holorusia sp.	2.0	60%	86
ODONATA			
Argia vivida Hagen	3.0	50%	56
Enallagma anna Williamson	1.4	50%	21

Flow rate of 1000 cc/min

Table 5

Long-Term Dissolved-Oxygen Bioassays Conducted

at the University of Utah (Minimum D.O. with Survival)

Species	Minimum D.O. level (mg/l)	% Survival	Survival time (days)
PLECOPTERA			
Acroneuria pacifica Banks	3.0	20%	41
Arcynopteryx parallela Frison	3.4	10%	8
	4.2	20%	28
Brachyptera nigripennis (Banks)	3.7	20%	9
Isoperla fulva Claassen	2.1	10%	27
Pteronarcella badia (Hagen)	2.0	30%	30
EPHEMEROPTERA			
Baetis bicaudatus Dodds	3.8	10%	3
Ephemerella grandis Eaton	3.5	50%	21
TRICHOPTERA			
Parapsyche elsis Milne	4.8	40%	16
DIPTERA			
Atherix variegata Walker	1.7	70%	90
Bibiocephala sp.	3.4	40%	21
ODONATA			
Argia vivida Hagen	1.7	10%	100 days
Enallagma anna Williamson	1.1	20%	35 days

Flow rate of 1000 cc/min

Table 6

Average Minimum Dissolved Oxygen Requirements

of Different Groups of Aquatic Invertebrates*

	Montana species	Average survival (days)	Utah species	Average survival (days)
Plecoptera	4.9 mg/l	62	2.8 mg/l	14
Ephemeroptera	4.6 mg/l	30	3.3 mg/l	10
Trichoptera	4.0 mg/l	85	3.1 mg/l	48
Diptera	2.4 mg/l	40	2.2 mg/l	92
Odonata			2.2 mg/l	39
Amphipoda	2.8 mg/l	20		

* Averages based on 50% + survival for time indicated.

The results of the longer term bioassays clearly indicate increased sensitivity and mortality of test specimens with increased length of exposure to low oxygen levels. For example, while 50% of the specimens of Acroneuria pacifica in Montana survived an oxygen concentration of 1.6 mg/l for 4 days, the minimal dissolved oxygen level for 50% survival at 111 days was 5.8 mg/l. Similarly, 50% of the specimens of Arcynopteryx aurea survived in an oxygen concentration of 3.3 mg/l for 4 days but only 30% survived at a dissolved oxygen level of 4.8 mg/l for 12 days. This increased sensitivity can be explained partly on the basis of physiological reactions such as debilitation due to lack of food and fungus infection. For example, 60% of the larvae of the crane fly, Holorusia sp., survived for 86 days at a dissolved oxygen level of only 2.0 mg/l. Shrinkage of the bodies of the larvae due to starvation and infection with fungus caused a rapid die-off after 86 days.

Of the eight species of aquatic insects tested at the Biological Station the carnivorous stonefly, Acroneuria pacifica, had the highest TLm with a 50% death rate at an oxygen level of 5.8 mg/l for 111 days. The most tolerant species was the Dipteran, Atherix variegata, with 90% of the specimens surviving for 40 days at an oxygen concentration of 2.4 mg/l. This species was also the most tolerant of the Utah forms tested with 90% of the specimens surviving at the same oxygen level for 97 days. The higher oxygen requirement of Acroneuria pacifica under long-term conditions may be partially

due to its food requirements. Inasmuch as this species is carnivorous, lack of a varied animal diet may have reduced its ability to tolerate low oxygen levels for extended periods of time.

A comparison of the long-term median tolerance limits of the same species of aquatic insects from Montana and Utah shows considerable variation. Fifty percent of the specimens of the stonefly, Acroneuria pacifica, from Montana died at a dissolved oxygen level of 4.4 mg/l in 69 days. The same percentage of Utah specimens survived at a much lower dissolved oxygen concentration, 3.0 mg/l, but for only 24 days. A mayfly, Ephemerella grandis, was tested from both Montana and Utah with similar results. Thirty percent of the Montana specimens survived at a dissolved oxygen level of 4.6 mg/l for 30 days while fifty percent of the Utah specimens survived at a dissolved oxygen concentration of 3.3 mg/l but for only 18 days. The differences in tolerance limits between the same species may have been much less if the tests had been conducted under exactly the same conditions in the two locations. Time did not permit this being done, so it was decided to run the Utah tests at lower oxygen levels in order to determine maximum survival rates at these much lower oxygen limits.

An evaluation of the average minimum dissolved oxygen requirements of the different groups of aquatic invertebrates tested shows the mayflies to be most sensitive, stoneflies next, and the caddis flies, fresh water shrimp, true flies, and damselfly, following in that order. While two species of mayflies could tolerate as low a dissolved oxygen concentration as 3.3 mg/l for 10 days, a level of 4.6 mg/l was required for 50% survival at 30 days. Three species of stoneflies from Utah survived at a dissolved oxygen concentration of 2.8 mg/l for 14 days with 50% surviving, but an average oxygen concentration of 4.9 mg/l was required for 30-50% survival for 62 days. The caddis flies tested also indicated higher oxygen levels were necessary with longer exposure with a minimum of 4.0 mg/l being required for 50% survival for 84 days.

The true flies, fresh water shrimp, and damselflies displayed a much greater tolerance than the previous three groups to low oxygen levels. Fifty percent of the specimens of these three groups were able to survive at dissolved oxygen levels ranging from 2.2 to 2.8 mg/l for periods ranging from 20 to 92 days.

While the principal objective of this project was to determine the minimal dissolved oxygen levels required for both short and long-term exposure, mere survival without growth and metamorphosis occurring would eliminate a species of aquatic insect eventually. While not all of the species tested molted or emerged during the study, many species did. All of the species on which bioassays were run for over 30 days molted one or more times at the oxygen levels required for 50% survival. Species such as the stoneflies, Brachyptera nigripennis, Pteronarcys californica, and Pteronarcella badia; the mayfly, Ephemerella grandis, and the damselfly, Enallagma anna, emerged

during the tests at oxygen concentrations of 4.8 mg/l or below. None of the caddis flies or Dipterans emerged inasmuch as only larvae and not pupae were used for testing purposes.

Discussion

Dissolved oxygen is an aquatic constituent which is rarely available in excess at all times. Many aquatic animals possess varied adaptations which facilitate the acquisition of oxygen when it becomes scarce. Diffusion, along with special ventilation mechanisms, provide extensive absorbing surfaces, in the case of stoneflies, for the absorption of oxygen from the environment. An adaption utilized by the nymphs of Pteronarcys californica, when environmental oxygen becomes reduced, is body undulations which attempt to destroy the oxygen gradient that develops around the body and gills. Of particular interest is the variation in the rate of these undulatory movements with year class. The undulations of the smaller nymphs of this species (year I, 17-18 mm long), in studies conducted at the University of Utah in 1963-65, were more rapid than that of the larger (year II, 30 mm long).

The respiratory mechanism possessed by different species of aquatic insects greatly influences their ability to withstand low oxygen concentrations. In work conducted by Knight and Gaufin (1966) at the University of Utah the value of gills in enabling some species to better withstand low dissolved oxygen levels was clearly demonstrated. The nymphs of Pteronarcella badia, Isoperla fulva, and Acroneuria pacifica were all exposed to an environment of reduced dissolved oxygen of 1.0 cc/l and water flow of 0.004 feet/second, at 10° C. The forms possessing gills exhibited quite similar mortalities during the exposure period. Pteronarcella badia nymphs exhibited a 13 percent mortality after 24 hours and 48 hours of exposure, and 29 percent at the end of 72 hours, with no further mortality for the remainder of the exposure period. Acroneuria pacifica showed the same mortality as Pteronarcella badia after 72 hours of exposure. After 96 hours exposure Acroneuria pacifica displayed a 25 percent mortality. No further mortality was noted for the remainder of the experimental period. Eighty percent of the Isoperla fulva nymphs, a species without gills, died within 24 hours. After 144 hours of exposure all had succumbed. The increased mortality shown by the Isoperla fulva nymphs may have been due to their smaller size and the fact that they were year class I, as opposed to year class II in the gilled forms. Isoperla fulva has only a one-year life cycle so it was impossible to compare nymphs of similar size.

In view of the above a second evaluation was carried out comparing nymphs of Acroneuria pacifica (gills) to those of Arcynopteryx parallela (no thoracic gills). The nymphs were tested at a temperature of 15.6° C with a water flow of 0.25 feet/second and a dissolved

oxygen concentration of 1.0 cc/l. The nymphs of both species were between 25 and 30 mm in length. In general, the results of this test, as in the case of the previous one, indicated that forms which lack gills are more sensitive to reduced dissolved oxygen than forms possessing gills. No mortality of Acroneuria pacifica nymphs occurred during the experimental period while nymphs of Arcynopteryx parallela showed an 82 percent mortality after 10 hours of exposure and 88.5 percent mortality at the end of 24 hours. After 34 hours of exposure all the nymphs were dead.

The metabolism of poikilotherms rises with temperature about two and one-half times per 10° C change in temperature (Prosser and Brown, ibid.). With this metabolic increase in response to increased environmental temperature, increased oxygen consumption results. The increase in oxygen consumption with increased water temperature would cause an aquatic insect subjected to the higher temperature (15.6° C) to incur an oxygen debt at a higher dissolved oxygen concentration than one subjected to a similar situation except exposed to a reduced temperature (10° C). Stoneflies, mayflies, and caddis flies do not have an apparent ability to get along without oxygen for an extended period. They do survive for a short period in greatly reduced oxygen by greatly reducing their activity, and they use energy apparently produced by the anaerobic phase of glycolysis. If the oxygen supply is not restored within a certain time, the specimens die from asphyxiation.

In the work conducted to date by the author and his colleagues there has been a great difference in the dissolved oxygen concentration at which initial mortality of test organisms was recorded. This difference was greatly influenced by the temperature difference in the experimental environment. In a natural situation resulting in the gradual reduction of dissolved oxygen over a short period of time due to intermittent discharges of organic oxygen-demanding wastes, the onset of stonefly mortality would be influenced by the existing water temperature. Providing the water flow and other variables remained constant, one could expect the aquatic insects subjected to an environmental temperature of 10° C to withstand reduced oxygen concentrations about 2.4 times lower than similar specimens exposed to a water temperature of 15.6° C. In a hypothetical situation, based on the work of Knight and Gaufin (1966), a stream possessing a temperature of 15.6° C and a dissolved oxygen concentration of 0.6 cc/l would have a stonefly mortality of 18 percent while a stream similar in all respects except possessing a water temperature of 10° C would exhibit 100 percent survival. Thus the water temperature of a stream is a very important factor in the survival of aquatic insects when they are subjected to a reduction in dissolved oxygen over a short period of time.

The rate of water flow in a stream also is a very important factor to be considered in the survival of aquatic insects when they are

exposed to low oxygen concentrations. Knight and Gaufin (1966) showed that a gradual reduction of dissolved oxygen with water flow of 0.06 ft/sec produced an approximate 50 percent stonefly mortality while a similar situation provided with a water flow of 0.25 ft/sec resulted in 100 percent survival.

In the present study the mean oxygen concentration required for 50% survival by 11 species of aquatic insects at a flow rate of 500 cc per minute was 3.64 mg/l. The mean for 10 species at a flow rate of 1000 cc per minute was considerably lower or 2.55 mg/l.

STUDIES ON THE
TOLERANCE OF AQUATIC INSECTS TO HEATED WATERS

Introduction

By 1980, it is estimated that around 200 billion gallons of cooling water will be needed daily, about one-sixth of the nationwide annual runoff, to meet projected steam electric power station needs based on once-through cooling (Pitcon, 1960). Water used for cooling purposes in industrial processes may be so hot and in such quantity that it may substantially raise the temperature of a receiving stream. Limited quantities of warm water, however, may produce desirable changes in selected localized situations. The requirements of the organisms in a stream must be known before realistic water quality standards can finally be adopted for their protection.

Literature concerning the effects of heated waters on aquatic insects is limited in extent and comparability. The effects of heated effluents on aquatic life have been reviewed in two recent comprehensive bibliographies, Mihursky and Kennedy (1967) and Raney and Menzel (1967). The effects of heated discharges on water quality and assimilation, aquatic organisms, and water uses have been thoroughly reviewed by Parker and Krenkel (1969). The temperature requirements of fish and other aquatic life were reviewed by Tarzwell (1968). Nebeker and Lemke (1968) tested the relative sensitivity of twelve species of aquatic insects to heated water in the laboratory. The lethal temperature at which 50% of the test specimens died after 96 hours exposure (TLm^{96}) ranged from 21 C for winter stoneflies to 33 C for dragonflies. An excellent review of temperature effects on aquatic insects was presented by Trembley (1965). Studies conducted by the Philadelphia Academy of Science (1968) on the effects of heated water on the insect fauna of the Potomac River have shown significant reductions in the diversity and numbers of organisms below a steam electric power plant. Coutant (1962) found substantial reductions in the volume and numbers of macroinvertebrates in the Delaware River in sections receiving heated water.

This section of the report summarizes the results of acute, short-term 96-hour tests (TLm^{96}) used in screening 15 species of aquatic insects to determine their relative sensitivity to heated water. The 96-hour TLm (Standard Methods, 1960) was used as a measure of effect in these tests. Long-term studies dealing with the effects of temperature on the reproduction, molting, emergence patterns, feeding rates, and long-term survival of aquatic insects were also conducted and will be considered in subsequent pages.

Materials and Methods

Test chambers consisted of oblong stainless steel tanks 90 cm long, 18 cm wide, and 17.5 deep. Similar tanks were utilized by Nebeker and Lemke (1968) in their studies on the tolerance of aquatic insects to heated waters at the National Water Quality Laboratory at Duluth, Minnesota. Fiberglass screening was employed to subdivide the tanks into three test cages 15 cm long, 17.5 cm wide, and 11 cm deep. Rocks were placed at the bottom of each cage to form a natural substrate for the aquatic organisms. The fresh water source was introduced at the forward end of the tank, which gradually slopes 7.5 cm to the overflow drain.

Five chambers were employed for temperature testing and one for a control, with the control maintained at the initial acclimation temperature. The oblong tanks were used as artificial streams where various water flows could be maintained with a stream of water and with paddle wheels.

The water used for all testing and for the holding tanks was obtained from the University of Montana Biological Station water system. This water originates in a spring, is chlorine-free, and has a constant temperature of 6.4 C± 0.1 C. The pH is 7.8± 0.1. Total hardness is near 135 ppm and the CO^2 varies from 1 to 2 ppm (CO_2 and total hardness expressed as ppm of $CaCO_3$). The dissolved oxygen level is consistently 100% of saturation or higher.

The test organisms, except for species of Simulium, Hexagenia, Atherix, and Gammarus, were collected from Rock Creek, a trout stream located southeast of Missoula, Montana. Simulium and Hexagenia were collected from Mud Creek, a slow flowing meadow creek, Atherix from the Clark's Fork of the Columbia River, and Gammarus from a spring-fed pond near Bigfork, Montana. All test organisms were mature larvae. The test organisms were placed in large, vigorously aerated, fiberglass holding tanks for a minimum of three days prior to testing. Fresh water was added at a rate of 3 to 5 liters per minute to insure a constant temperature and a fresh water supply.

Desired temperatures in the test chambers were obtained by manual regulation of mixing faucets. Temperatures were allowed to stabilize over a period of 24 hours to insure uniformity. If the system remained stable during this 24-hour period, the test was initiated.

Experimentation began with an initial series of temperatures usually ranging from 10 to 25 C. The specimens were placed in an aerated water bath, and the temperature gradually raised (2 to 4 C per hour) to the appropriate test temperature before they were transferred to the appropriate test chambers. This procedure was followed to insure against nebulous results induced either by thermal "shock" from immediate transfer from one temperature to another or by the complete acclimation that can accompany a very gradual increase in temperature.

In the test chambers the paddle wheels created a turbulence and helped maintain a dissolved oxygen level of 100% saturation or higher. A liberal fresh water supply was provided (at least 2 liters per minute) for the removal of toxic waste. Temperature values were taken at least four times daily and if any value varied by more than 0.5 C, the test was discarded. If any of the control organisms died, the test was terminated.

The temperature at which 50% of the organisms died was obtained by a modification of the straight line graph interpolation method as outlined in Standard Methods (1960).

Results

Late instar larvae of 15 species of aquatic insects and one species of amphipod were tested to determine their tolerance of high water temperatures. A marked difference in sensitivity was apparent (Table 7) in the different species. A mayfly, Cinygmula par Eaton, died at 11.7 C and was the most sensitive of all the species tested. This species is found in very cold clear mountain streams in Montana. The fresh water shrimp, Gammarus limnaeus Smith, proved to be surprisingly sensitive to temperature increases, exhibiting a 96-hour TLm of only 14.5 C. Ephemerella doddsi Needham, a small, widely distributed mayfly characteristic of cold turbulent streams in the Intermountain Region, was also very sensitive with a TLm value of 15.4 C. A lotic species of mayfly, Hexagenia limbata Guerin, was much more tolerant than other mayflies tested with a TLm of 26.6 C.

Considerable difference in susceptibility to temperature increases existed between the three species of stoneflies tested. Isogenus aestivalis (Needham and Claassen) was quite sensitive, 50% dying at 16 C, while Pteronarcella badia (Hagen) and Pteronarcys californica Newport, two closely related species, survived increases to 24.6 and 26.6 C respectively. Six species of caddis flies were tested and clearly reflected thermal differences in their habitat requirements. Parapsyche elsis Milne, which is largely restricted to cold, fast flowing mountain streams, had a TLm of 21.8 C while Hydropsyche sp. taken from a slow flowing stream draining a marshy lake was very tolerant with a TLm of 30.1 C. Atherix variegata Walker, the snipe fly, was the most tolerant of all species tested with a TLm of 32.6 C. No dragonfly or damselfly nymphs were tested because a thick ice and snow cover coating their habitats early in the winter prevented collecting large enough numbers for testing purposes.

Discussion

The rate of development and the time of emergence of aquatic insects is directly influenced by the temperature. An increase in water temperatures in the winter above 5 C might completely eliminate

winter stoneflies belonging to the family Capniidae.

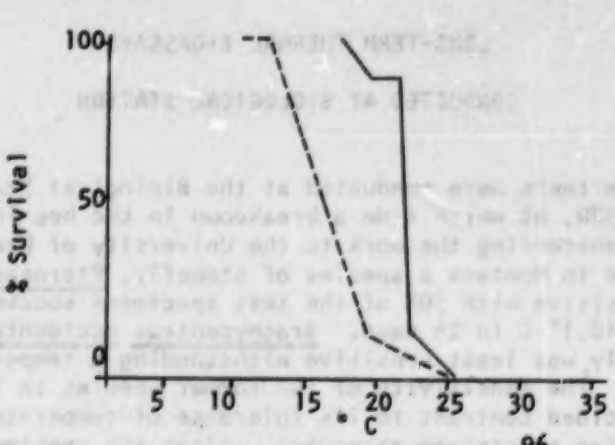
Many species of stoneflies, mayflies, and caddis flies emerge in late spring before stream temperatures reach high summer levels. An artificial increase in stream temperatures during the winter would very likely cause these species to develop more rapidly, emerge earlier, and be killed by cold air temperatures, and may substantially reduce the population or eliminate the species.

The stonefly Isogenus aestivalis and mayfly Cinygmula par are largely restricted to clear, cold water streams in the Intermountain Region and even a slight increase in water temperature may have an adverse effect on their survival. By comparison the snipe fly, Atherix variegata, is often found in open sections of streams which warm up during the summer months and this species is decidedly temperature tolerant.

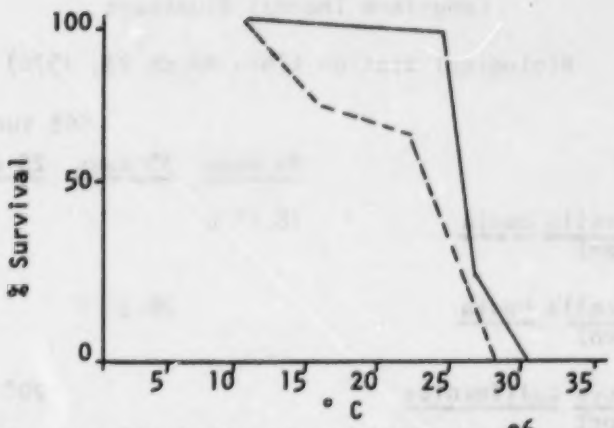
Two of the species of stoneflies tested, Pteronarcella badia and Pteronarcys californica, are common in medium to large streams in the western United States and are comparatively temperature tolerant. These species require two and three years respectively to complete their life cycle and have become adapted to the warmer waters of late summer which many aquatic insects avoid by emerging in the spring.

Table 7. Temperatures (°C) at which 50% of the test species died after 96 hours exposure (TLm^{96}), Bigfork, Montana, 1968-69

Species tested	Test 1	Test 2	Test 3	Mean TL_3	Average group TLm
DIPTERA					
Atherix variegata Walker	32.6	32.2	....	32.4	28.7
Simulium sp.	25.0	25.2	....	25.1	
TRICHOPTERA					
Parapsyche elsis Milne	21.8	21.6	....	21.7	
Limnephilus ornatus Banks	24.5	25.0	....	24.75	
Neothrema alicia Banks	25.8	26.0	....	25.9	26.5
Drusinus sp.	27.2	27.4	....	27.3	
Brachycentrus occidentalis Banks	29.7	....	....	29.7	
Hydropsyche sp.	30.0	30.1	....	30.05	
PLECOPTERA					
Isogenus aestivalis (Needham and Claassen)	16.0	16.3	....	16.15	
Pteronarcella badia (Hagen)	24.4	24.6	24.2	24.4	22.55
Pteronarcys californica Newport	28.0	26.4	26.6	27.0	
EPHEMEROPTERA					
Cinygmula par Eaton	11.7	....	....	11.7	
Ephemerella doddsi Needham	15.4	15.5	....	15.45	18.82
Ephemerella grandis Eaton	21.5	....	....	21.5	
Hexagenia limbata Guerin	26.1	27.1	....	26.6	
AMPHIPODA					
Gammarus limnaeus Smith	14.5	14.6	....	14.55	14.55



Ephemerella doddsi, dashed line, TLm^{96} = 15.5 C
Ephemerella grandis, solid line, TLm^{96} = 21.5 C



Pteronarcella badia, dashed line, TLm^{96} = 24.6 C
Pteronarcys californica, solid line, TLm^{96} = 26.6 C

Fig. 2 Straight-line interpolation graphs of representative TLm^{96}'s

LONG-TERM THERMAL BIOASSAYS

CONDUCTED AT BIOLOGICAL STATION

Long-term tests were conducted at the Biological Station through March, 1970, at which time a breakdown in the heating system necessitated transferring the work to the University of Utah. In the work conducted in Montana a species of stonefly, Pteronarcella badia, was most sensitive with 50% of the test specimens succumbing to a temperature of 18.1° C in 24 days. Brachycentrus occidentalis, a case making caddis fly, was least sensitive withstanding a temperature of 26° C for 45 days. The sensitivity of the former species to longer term exposure was a decided contrast to its tolerance of temperatures as high as 24.6° C for short-term exposures. Since the specimens involved in the longer term tests were collected during the winter months, it is possible that acclimation to low winter temperatures increased the sensitivity of the specimens tested. The tolerance of the four species tested is summarized in the following Table 8.

TABLE 8

Long-Term Thermal Bioassays

Biological Station (Thru March 23, 1970)

	50% Survival				
Species	24 days	30 days	25 days	45 days	12 days
Pteronarcella badia (Hagen)	18.1° C				
Pteronarcella badia (Hagen)		20.5° C			
Pteronarcys californica Newport			20° C		
Brachycentrus occidentalis Banks				26° C	
Ephemerella grandis Eaton					21.5° C

Specimens of Pteronarcys californica clearly showed the effects of exposure to higher temperatures on their developmental rate. This species normally emerges in Montana streams in mid June. Three specimens emerged on January 5, 1970, after being exposed to a temperature of 18.4° C for 25 days.

STUDIES ON THE TOLERANCE OF GREAT BASIN AQUATIC INSECTS TO HEATED WATERS

Acute, short-term 96-hour tests were also conducted at the University of Utah during 1970 with 8 species of aquatic insects to determine their relative sensitivity to heated water. Longer term studies were also conducted to determine the long-term survival of 16 species of aquatic insects and the effects of elevated temperatures on their molting and emergence patterns.

Materials and Methods

Test chambers consisted of stainless steel tanks 36 inches long, 7 inches wide, and 7 inches deep. These were immersed in two large refrigerated water baths. Eight of these tanks were used for screening temperatures ranging from 14.5° C to 29° C. A ninth tank was used as a control with a temperature of 10° C. A stainless steel 700 watt National Appliance Company heater was placed in each tank for raising the water temperature to the desired level. The temperature in each tank was controlled by a National Appliance Company thermostatic unit. A paddle wheel was used for circulating the water in each test chamber.

The water used for all testing and for the holding tank was obtained from artesian wells which supply the University of Utah with culinary water. The water is non-chlorinated and varies little chemically throughout the year. The dissolved oxygen content varies between 7.0 to 9.0 ppm; CO_2 between 0 - 1 ppm; pH 7.8 - 8.2; carbonates 0.0; and bicarbonates 165.0 to 225.0 ppm.

The test organisms were collected from streams in the Wasatch and Uintah Mountains within a radius of 50 miles from the University of Utah. All organisms tested were mature larvae. The specimens were maintained in a large, vigorously aerated, fiberglass holding tank for a minimum of three days prior to testing. In conducting the tests 20 specimens of each species were held in small fish breeder nets suspended in each test chamber. A fresh water supply of approximately 2 liters per minute was provided for the removal of toxic wastes. Temperature readings were taken several times daily with any variation being maintained at ± 1.0° C. A YSI Model 47 Scanning Tele-Thermometer was used for recording temperatures.

Results

A marked difference in the sensitivity of the various species tested was apparent in both the acute and long-term studies. The mayfly, Ephemerella doddsi, died at 16.0 C in 96 hours and was the most sensitive of the species tested. This value was close to the 96-hour TLm of 15.4 C obtained for the same species in Montana. The snipe fly, Atherix variegata, was the most tolerant species tested with all specimens surviving for 96-hours at a temperature of 29.0 C. This corresponded to the 96-hour TLm of 32.6 C obtained with Montana specimens. However, specimens of the stonefly, Isogenus aestivalis, from Utah were much more tolerant than Montana specimens with a 96-hour TLm of 24.2 C in comparison with a 96-hour TLm of 16.1 C for the latter specimens. Acclimation to the colder temperatures encountered in Montana streams may account for the difference. The results of the 96-hour tests conducted at the University of Utah are given in Table 9.

Long-term thermal bioassays were conducted with 16 species of aquatic insects with all species showing increased sensitivity with time of exposure. (Table 10). Bibiocephala grandis, a Dipteran, found only in cold torrential streams of the Intermountain Region, was the most sensitive species with only 60% survival after 3 days at a temperature of 15 C. Atherix variegata, the snipe fly, and Brachycentrus occidentalis, a caddis fly, were the most tolerant with 50% of the specimens surviving at 28 C for 46 days and 14 days respectively.

Emergence

The effect of elevated temperatures on growth rate and time of emergence was clearly shown by the research conducted at the University of Utah. Six species emerged in the laboratory prior to the natural period of emergence found in the region. Five Plecoptera and one Odonate emerged early in response to increased temperature. The organisms were primarily affected by the length of the exposure period and the temperature level. Each organism reacted in a pattern dissimilar to the emergence of the other species.

Acroneuria pacifica began to emerge approximately three months prior to its normal period. The first four specimens emerged on April 12, 1971, after 4 weeks at 18° C. A total of nine specimens emerged.

The first Arcynopteryx parallela emerged at 15° C on February 16, 1971, after being exposed for six weeks. Emergence commenced approximately two months early. The activity increased in intensity with the longevity of exposure. Twenty-three adults emerged over a nine-week period.

Arcynopteryx signata started to emerge five days after being subjected to 18° C. The first adult appeared on April 24, 1971, approximately one month prior to normal emergence activity. A total of nine specimens emerged over a period of 2.5 weeks.

After 2.5 weeks exposure to 20° C, Isoperla fulva began emergence on April 6, 1971. Six specimens emerged over a five-week period.

Initial emergence of Pteronarcella badia occurred on February 2, 1971, at 15° C four months prior to the normal emergence period. This organism was subjected to heated water for a period of 4 weeks prior to adults appearing. Emergence continued over a fifteen-week period with a total of forty-seven specimens emerging.

Argia vivida began emerging on April 12, 1971, after 3.5 weeks at 24° C. Normal emergence in this region occurs in early June. Twenty-nine adults emerged over a four-week period.

Table 9. Temperatures (°C) at which over 50% of the test species survived after 96 hours exposure (TLm^{96}), University of Utah, Salt Lake City, Utah, 1970.

Species tested	% Survival	Temperature	
PLECOPTERA			
Acroneuria pacifica Banks	70%	27.0	
Isogenus aestivalis (Needham and Claassen)	50%	24.2	
Arcynopteryx parallela Frison	70%	23.0	
	45%	18.0	(Winter test)
EPHEMEROPTERA			
Ephemerella doddsi Needham	50%	22.0	
	60%	16.0	
TRICHOPTERA			
Brachycentrus occidentalis Banks	60%	29.0	
	70%	28.0	
Arctopsyche grandis (Banks)	40%	20.0	
DIPTERA			
Atherix variegata Walker	100%	29.0	
Holorusia grandis	80%	26.0	
	0%	28.0	

Table 10

Long-Term Thermal Bioassays

University of Utah

1970-71

Species tested	Temperature	Exposure time (days)	% Survival
PLECOPTERA			
Acroneuria pacifica Banks	15° C	31	50%
Arcynopteryx signata (Hagen)	15° C	14	60%
Arcynopteryx parallela Frison	15° C	41	55%
Brachyptera nigripennis (Banks)	14.5° C	5	50%
Isoperla fulva Claassen	18° C	11	40%
Pteronarcella badia (Hagen)	17.5° C	38	45%
EPHEMEROPTERA			
Ephemerella grandis Eaton	17.5° C	18	50%
Rhithrogena robusta Dodds	15° C	4	50%
TRICHOPTERA			
Arctopsyche grandis (Banks)	18.0° C	23	50%
Brachycentrus occidentalis Banks	28.0° C	14	45%
Parapsyche elsis Milne	15.0° C	14	40%
Rhyacophila fuscula	15.0° C	39	40%
DIPTERA			
Biblocephala grandis	15.0° C	3	60%
Atherix variegata Walker	28° C	46	50%
Holorusia grandis	24° C	31	45%
ODONATA			
Argia vivida	18° C	29	50%

STUDIES ON THE TOLERANCE OF AQUATIC INSECTS TO LOW pH

Introduction

In coal mining regions of the United States water pollution by acid mine drainage constitutes a problem of major importance. Drainage from many bituminous coal mines contains large quantities of sulfuric acid as the result of the chemical and biological oxidation of sulfur compounds associated with the coal seams. Streams receiving such drainage may have a pH as low as 3.0 to 4.0. Pollution by acids may be sufficient to not only make the water of a receiving stream unfavorable for the growth and development of fish and aquatic invertebrates but there may also be a directly lethal effect.

Numerous field studies have demonstrated the deleterious effects of acid mine drainage on receiving waters. Lackey (1939) reported that the number of species of microscopic forms in any given habitat at or below a pH of 3.9 was very small. Parsons (1956) found a relatively small number of benthic invertebrates in a central Missouri stream below an acid strip mine. Harrison (1958) found a very restricted flora and fauna in a stream near Johannesburg, South Africa, in which the pH was between 3.7 to 4.3. A dramatic indication of the effects of acid mine pollution in Pennsylvania occurred in July, 1964, in the form of a massive fish kill in Slippery Rock Creek. Flushing out of pockets of mine acid from strip cuts and abandoned deep mines following a heavy rainfall killed thousands of fish and invertebrates in a receiving stream.

A review of the literature revealed that few laboratory studies have dealt with the effects of low pH on the biota of streams, particularly with the bottom fauna. Stickney (1922) conducted a series of laboratory experiments on the relation of a species of dragonfly to acid and temperature.

Research by Jewell (1922) indicated that fish can live in water having a minimum pH of 4.4 with a pH of 4.3 being lethal. Bell and Nebeker (1969) tested 10 species of aquatic insects and obtained TLm^{96} values ranging from pH 4.65 for mayflies to pH 1.5 for caddis flies.

This report summarizes the results of acute short-term 96-hour tests (TLm^{96}) and long-term continuous flow tests used in screening 19 species of aquatic invertebrates to determine their relative tolerance to low pH. The 96-hour TLm (Standard Methods, 1960) was used as the measure of effect in these tests. Further long-term studies dealing with the effects of low pH on factors such as molting, adult emergence, reproduction, and long-term survival are being conducted. The test species included the stoneflies, Acroneuria pacifica Banks, Arcynopteryx

parallela Frison, Isogenus aestivalis (Needham and Claassen), Pteronarcys californica Newport, Pteronarcella badia (Hagen); mayflies, Cinygmula par Eaton, Ephemerella grandis Eaton, Heptagenia sp., Ephemerella doddsi Needham, Hexagenia limbata Guerin, Rithrogena robusta Dodds, Leptophlebia sp.; caddis flies, Brachycentrus occidentalis Banks, Cheumatopsyche sp., Hydropsyche sp.; true flies, Atherix variegata Walker, Simulium vittatum Zetterstadt, and fresh water shrimp, Gammarus limnaeus Smith.

Materials and Methods

All tests were conducted in fiberglass tanks measuring 252 cm long, 21 cm wide, and 25 cm deep. The tanks were partitioned with glass plates into six test chambers each, measuring 36 cm long, 21 cm wide, and 16 cm deep. These chambers were further subdivided into three test cages measuring 13 cm long, 21 cm wide, and 16 cm deep. Each chamber was furnished with a glass overflow tube capped with a fiberglass plug to prevent the loss of any test organisms. Rocks were placed in the bottom of the cages to form a natural substrate for the organisms.

Concentrated sulphuric acid was used exclusively in all tests. A proportional diluter (Mount and Brungs, 1967) was used to deliver the solutions of various pH to the test chambers. Mixing tanks were added to the diluter to insure thorough mixing of the acid and water prior to delivery to the test chambers. A mariotte bottle was used to deliver the 10:1 solution of acid and water to the diluter. Prior to each test the diluter was cycled for 24 hours to insure stabilization. If, at the end of the 24-hour cycling period, no malfunctions occurred, the test was initiated.

The test organisms, with the exception of Gammarus, Simulium, and Leptophlebia, were collected from Rock Creek, a trout stream located southeast of Missoula, Montana. Simulium and Leptophlebia were collected from Mud Creek, a small slow-flowing stream located north of the Biological Station in Flathead County, Montana. Specimens of Gammarus were collected from a spring fed, heavily vegetated pond located near Bigfork, Montana. All test organisms used were mature larvae or nymphs. The organisms were held in large, vigorously aerated fiberglass holding tanks for a minimum of three days prior to testing. Fresh water was added to the holding tanks at a rate of three to five liters per minute to insure constant temperature and fresh water supply.

The water used for all tests and for the holding tanks was obtained from the Biological Station water system. This water originates in a spring; is chlorine free; is constant at 6.4 degrees C and pH of 7.8± .1; and is chemically stable. The total hardness remains consistently near 135 ppm; the CO_2 from 1 to 2 ppm; (CO_2 and hardness expressed as $CaCO_3$). Dissolved oxygen levels remain constantly at 100% saturation or greater.

In all tests, pH values in the 2, 3, 4, 5, and 6 ranges were used. The acclimation pH of 7.8± .1 was used as the control pH value. The water in each test chamber was aerated to insure dissolved oxygen saturation and to create turbulence. The diluter was calibrated to cycle every three minutes to insure a liberal fresh water supply and a constant temperature of 9.5° C. The test organisms were transferred immediately from the holding tanks into the test cages. No attempt was made to decrease pH values down to the test value to prevent shock.

During the test period, pH values were recorded four times daily with a Corning Model 12 pH meter. If any of the pH values varied by more than .25 pH units the test was discarded. If any of the organisms at the control pH died, the test was also discarded. All tests, except that with Rhithrogena, were duplicated. Tests with Gammarus were quadruplicated.

The pH values at which 50% of the test organisms died were obtained by using a modification of the straight line graph interpolation method as outlined in Standard Methods (1960). The mean of each duplicate test was plotted as the final TLm_{50} value for each test organism.

Results

Late instar larvae and nymphs of 19 species of aquatic invertebrates were tested to determine their relative tolerance to low pH. Tables 11 and 12 show that considerable difference in tolerance occurs between the different species. In comparison with the results obtained by Bell and Nebeker (1968) on 11 species of aquatic insects from Minnesota the TLm^{96} are decidedly higher. This may be due to acclimation to differences in pH in the streams from which the species were obtained. The minimum pH encountered in the Montana streams from which the test specimens were collected was 6.8 while in Minnesota much more acid streams exist. The caddis fly, Limnephilus ornatus, was the most tolerant species tested with a 96-hour TLm of 2.83 while the amphipod, Gammarus limnaeus, was very sensitive with a 96-hour TLm of 7.27. Simulium sp. proved to be moderately tolerant with a TLm^{96} of 3.64. All four species of stoneflies tested, Pteronarcella badia, Pteronarcys californica, Arcynopteryx parallela, and Isogenus aestivalis are moderately tolerant with TLm^{96} values of 4.37, 4.6, 5.33, and 5.24 respectively. It is interesting to note that the sensitivity of Pteronarcys californica compares closely with that of Pteronarcys dorsata tested by Bell and Nebeker. The latter species had a TLm^{96} of 4.25. The mayflies tested proved to be more sensitive than the stoneflies with Ephemerella doddsi being most tolerant with a TLm^{96} of 5.13 and Rhithrogena robusta being least tolerant with a TLm^{96} of 6.35.

The results of long-term continuous flow bioassays confirmed the relative sensitivity of the orders of aquatic insects tested with the mayflies being most sensitive, the stoneflies moderately sensitive,

and the caddis flies least sensitive. The number of deaths of each species, however, increased with time of exposure with 50% of the specimens of such species as Acroneuria pacifica succumbing within 90 days and a like percentage of Ephemerella grandis dying within 68 days.

Discussion

In general, the test organisms died at pH values below those normally found in the field. While numerous papers have been published dealing with the hydrogen ion concentration of natural waters, the role of pH in fresh water ecology is still something of a mystery. Some ecologists have maintained that pH of natural waters is a supreme controlling factor determining the presence and distribution of aquatic organisms but this viewpoint is not generally recognized. However, it is fairly clear that acids can affect aquatic insects by bringing about changes in the conditions of existence and rate of growth, by being directly lethal if present at high enough concentrations, and by being harmful because they have anions of high toxicity or by having marked toxic properties as undissociated molecules.

The mayflies which were tested in this study were less tolerant than the genera reported by Bick (1953). He listed the genera Stenonema, Baetis, Blasturus, Callibaetis, and Paraleptophlebia, as being present in streams having pH values of 4.0 to 5.0. While the first 4 genera are found in Montana, the species of mayflies tested in the present study are more characteristic of fast flowing, cold mountain streams fed by snow melt or springs. Hydrogen ion concentrations as low as Bick reported are very unlikely to occur under such conditions.

The stoneflies tested were moderately tolerant to low pH values but a pH of 4.5 would undoubtedly eliminate them completely on long-term exposure. Leuctra nymphs have been collected in Glacier National Park in a small stream feeding McGee's meadow at pH values of 6.7 to 6.8. A decline in pH to 6.0, however, was at least partially responsible for eliminating this species lower in the stream.

Of the caddis flies tested, Limnephilus ornatus was most tolerant with a 96-hour TLm of 2.83. While this species was taken from a slow moving stream with a pH of 7.2 or above, closely related members of the family Limnephilidae often occur in acid bogs. The TLm^{96} value of 3.35 for Hydropsyche sp. corresponds closely with the pH values of 4.0 to 5.7 reported by Bell and Nebeker (1959) for Hydropsyche betteni. The case making caddis fly, Brachycentrus occidentalis, which had a 90-day TLm of 4.5, while moderately tolerant, was much more sensitive than Brachycentrus americanus tested by Bell and Nebeker (TLm^{96} pH 1.5). Acclimation is probably responsible for this difference with B. occidentalis occurring in streams in Montana with a pH of 7.8 or above while the latter species occurs in distinctly acid streams in Minnesota.

The results of the bioassays indicate that the species tested can live for short periods of time at pH values below those normally found in the field. Longer exposure, however, may have decidedly detrimental effects on molting, growth, and reproduction as well as survival for longer periods of time.

Table 11. pH values at which 50% of the test species died after 96 hours exposure (TLm^{96}), Flathead Lake, Montana, 1968-69

Species tested	pH Values	Mean
EPHEMEROPTERA		
Ephemerella doddsi Needham	4.95	5.13
	5.35	
Leptophlebia sp.	5.30	5.21
	5.11	
Hexagenia limbata Guerin	6.40	5.90
	5.40	
Cinygmula par Eaton	6.25	6.04
	6.00	
Rhithrogena robusta Dodds	6.35	6.35
Heptagenia sp.	6.25	6.18
	6.11	
PLECOPTERA		
Arcynopteryx parallela Frison	5.50	5.33
	5.16	
Pteronarcys californica Newport	5.12	4.60
	4.19	
Pteronarcella badia (Hagen)	4.90	4.37
	4.19	
Isogenus aestivalis (Needham and Claassen)	5.40	5.15
	4.90	
TRICHOPTERA		
Limnephilus ornatus Banks	2.72	2.83
	2.94	
Hydropsyche sp.	3.60	3.34
	3.10	
DIPTERA		
Simulium vittatum Zetterstadt	3.68	3.64
	3.59	
AMPHIPODA		
Gammarus limnaeus Smith	7.31	7.29
	7.28	
	7.20	7.27
	7.34	

Table 12. pH values at which 50% of the test species died after long-term continuous exposure

Species tested	Exposure time			
	90 day TLm	68 day TLm	48 day TLm	33 day TLm
EPHEMEROPTERA				
Ephemerella grandis Eaton			5.8	
Hexagenia limbata Guerin				5.5 70 of 90 survived
PLECOPTERA				
Acroneuria pacifica Banks	5.8			
Pteronarcys californica Newport	4.95			
Pteronarcella badia (Hagen)	4.52			
TRICHOPTERA				
Brachycentrus occidentalis Banks	4.3			
Cheumatopsyche sp.	4.52			
DIPTERA				
Atherix variegata Walker		4.2		

TOLERANCE LIMITS OF GREAT BASIN AQUATIC INSECTS TO SULFURIC AND HYDROCHLORIC ACID

All three phases of this project dealing with the water quality requirements of aquatic insects were transferred from the Montana Biological Station to the University of Utah in June, 1970. This proved to be advantageous in extending the scope of the work to include other species of insects and water with different chemical characteristics. The research conducted at the University of Utah showed significant differences in sensitivity between some of the species which were tested in both locations.

Materials and Methods

Bioassays were conducted in a constant temperature room where the test species were first retained in untreated water while acclimatizing to laboratory conditions before being transferred to the bioassay aquaria. All temperatures were controlled thermostatically at 8° C.

The bioassay equipment consisted of 12 glass aquaria with approximately two gallon capacities each. Acid concentrations ranging from pH 2.0 to 6.0 were used in the experiments.

Each acid was tested separately using non-exposed specimens for each bioassay and all tests were conducted in duplicate for 96-hour periods.

Test specimens were observed at 24-hour intervals and recorded as the number unaffected, the number affected, and the number dead.

Results

A wide range in pH existed for both acids with TLm^{96} values ranging from pH 2.8 to 5.7 in sulfuric and pH 2.7 to 5.6 in hydrochloric acid.

The average of the combined response from the duplicated tests illustrated a similar lethal effect of the acids at most pH values among the majority of the test species. A difference, however, was noted in the sub-lethal response where many specimens exhibited a more noticeable change in behavior and response to sulfuric acid than was observed in the hydrochloric acid tests.

The calculated TLm^{96} values showed *Holorusia* spp. most tolerant to sulfuric acid with TLm^{96} at pH 2.8 and *Arctopsyche grandis* most tolerant to hydrochloric acid with TLm^{96} at 2.7. The least tolerant species was *Gammarus lacustris* to both acid solutions with TLm^{96} at pH 5.7 in sulfuric and TLm^{96} at pH 5.6 in hydrochloric acid.

The species used in the study adapted to laboratory conditions within a short period of time and molting was observed among many of the specimens. Near the end of the study many of the mature nymphs of Ephemerella grandis grandis displayed signs of emergence and before the bioassays were completed some emerged as adults.

The bioassays are indicative of short-term exposure under ideal laboratory conditions. The response produced is due to the influence of the acids without the consideration of other stress factors that could result from conditions in the natural environment.

Table 13

TLm^{96} Values for Sulfuric Acid Bioassays

	pH	Acid moles/liter
Holorusia spp.	2.8	1.6×10^{-3}M
Arctopsyche grandis	3.4	3.9×10^{-4}M
Ephemerella grandis grandis	3.6	2.5×10^{-4}M
Pteronarcella badia	3.7	2.0×10^{-4}M
Acroneuria pacifica	3.8	1.6×10^{-4}M
Ephemerella doddsi	3.8	1.6×10^{-4}M
Arcynopteryx parallela	4.1	8.0×10^{-5}M
Rhithrogena robusta	4.3	5.0×10^{-5}M
Isoperla fulva	4.5	3.3×10^{-5}M
Gammarus lacustris	5.7	2.0×10^{-6}M

Table 14

TLm^{96} Values for Hydrochloric Acid Bioassays

	pH	Acid moles/liter
Arctopsyche grandis	2.7	2.0×10^{-3}M
Holorusia spp.	3.2	6.3×10^{-4}M
Acroneuria pacifica	3.6	2.5×10^{-4}M
Ephemerella grandis grandis	3.7	2.0×10^{-4}M
Ephemerella doddsi	3.8	1.6×10^{-4}M
Rhithrogena robusta	4.1	8.0×10^{-5}M
Pteronarcella badia	4.4	3.9×10^{-5}M
Arcynopteryx parallela	4.6	2.5×10^{-5}M
Isoperla fulva	4.6	2.5×10^{-5}M
Gammarus lacustris	5.6	2.5×10^{-6}M

Long-Term Continuous Bioassays

Long-term continuous flow bioassays were initiated at the University of Utah in January, 1971, utilizing the same methods and the equipment used at the Montana Biological Station. The test species included the stoneflies, Acroneuria pacifica Banks, Arcynopteryx parallela Frison, and Pteronarcella badia (Hagen); mayflies, Ephemerella doddsi Needham, Ephemerella grandis Eaton, and Rhithrogena robusta Dodds; caddis flies, Arctopsyche grandis (Banks), Brachycentrus occidentalis Banks; and Rhyacophila sp.; and the damsel fly, Argia sp. The mayfly, Rhithrogena robusta, a species found only in cold, clear, well aerated streams in the Intermountain Region, was most sensitive with all specimens dying at a pH of 5.7 and only two surviving at pH 6.1, after 12 days. The stonefly, Acroneuria pacifica, was moderately tolerant with 4 of 10 specimens surviving at a pH 6.1 for 50 days.

Table 15

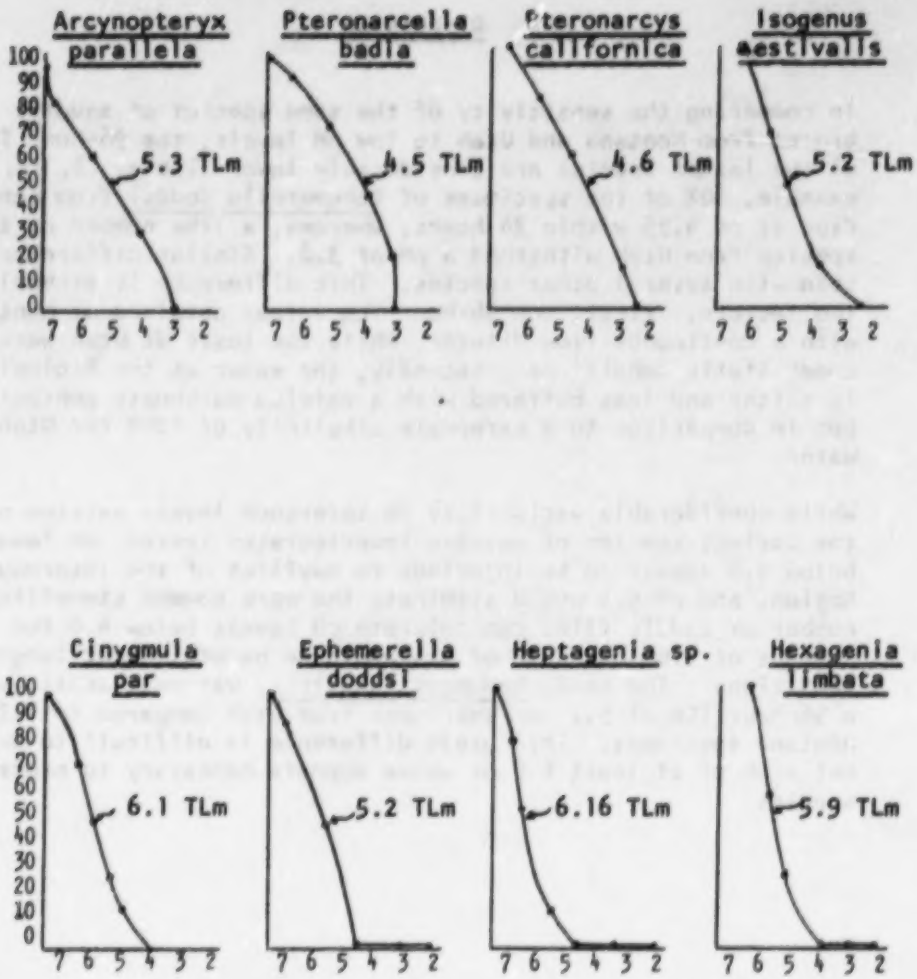
Long-Term Bioassay Results at Low pH

Species tested	pH range No. surviving				Control	Exposure time
	4.5	5.0	5.6	6.0		
EPHEMEROPTERA						
Ephemerella doddsi Needham	0	0	1	3	10	16 days
	4.6	5.1	5.7	6.1		
Ephemerella grandis Eaton	0	0	3	4	10	26 days
	4.6	5.1	5.7	6.1		
Rhithrogena robusta Dodds	0	0	0	2	10	12 days
ODONATA						
	3.0	3.4	4.1	6.1		
Argia vivida	2	6	8	10	10	16 days, Bio. not completed
PLECOPTERA						
	4.5	5.0	5.6	6.1.		
Acroneuria pacifica Banks	0	1	1	4	10	50 days
	4.5	5.1	5.6	6.1		
Arcynopteryx parallela Frison	0	0	1	0	0	43 days
			1 emer.	7 emer.	10 emer.	
	3.3	3.8	4.3	6.1		
Pteronarcella badia (Hagen)	0	0	2	7	10	33 days
TRICHOPTERA						
	3.1	3.4	4.2	6.1	10	43 days
Arctopsyche grandis (Banks)	0	0	4	8		
	3.1	3.4	4.2	6.1	10	39 days
Brachycentrus occidentalis Banks	0	0	2	4		
	3.1	3.4	4.2	6.1	10	30 days
Rhyacophila fuscula	0	0	1	3		

Discussion

In comparing the sensitivity of the same species of aquatic invertebrates from Montana and Utah to low pH levels, the 96-hour TLm values of the latter species are considerably lower (Tables 13, 14, 15). For example, 50% of the specimens of Ephemerella doddsi from Montana died at pH 4.95 within 96 hours; whereas, a like number of the same species from Utah withstood a pH of 3.8. Similar differences can be seen with several other species. This difference is probably due to two factors. First, the 96-hour TLm values obtained at Montana were with a continuous flow diluter, while the tests at Utah were conducted under static conditions. Secondly, the water at the Biological Station is softer and less buffered with a calcium carbonate content of 135 ppm in comparison to a carbonate alkalinity of +200 for Utah well water.

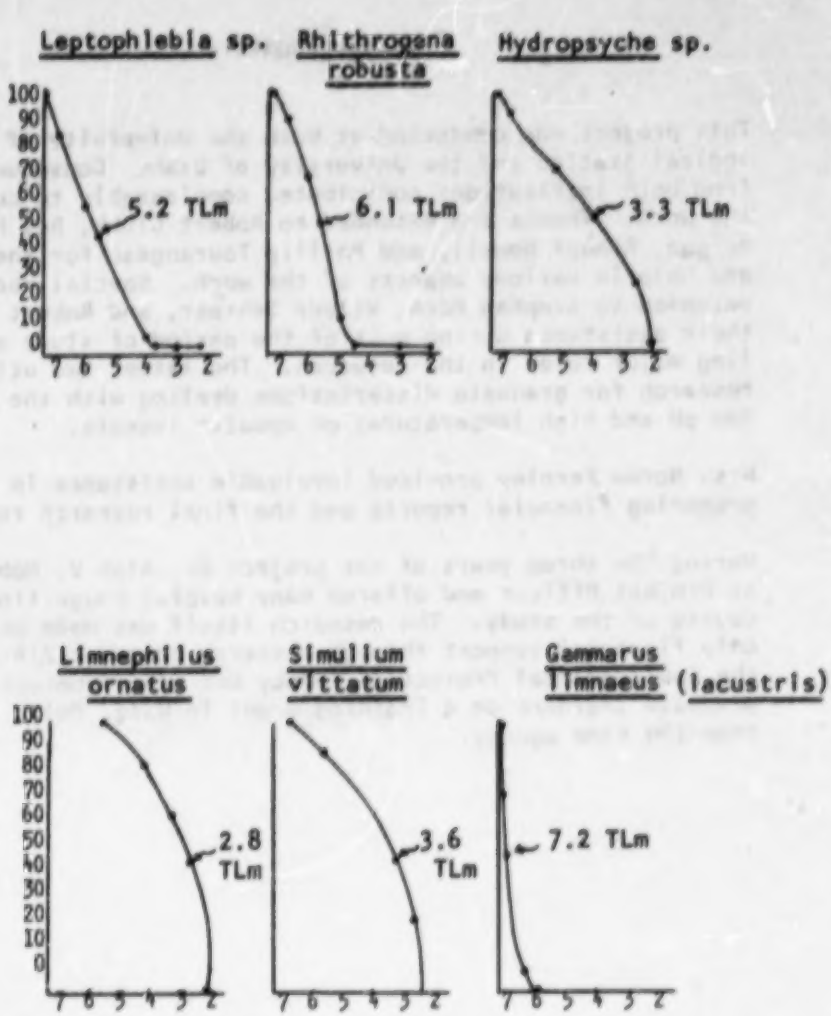
While considerable variability in tolerance levels existed between the various species of aquatic invertebrates tested, pH levels below 6.0 appear to be injurious to mayflies of the Intermountain Region, and pH 5.5 would eliminate the more common stoneflies. A number of caddis flies can tolerate pH levels below 4.0 for short periods of time but a pH of 4.5 would be harmful under long-term conditions. The scud, Gammarus lacustris, was most sensitive with a 96-hour TLm of 5.7 for specimens from Utah compared to 7.27 for Montana specimens. This great difference is difficult to explain but a pH of at least 6.0 or above appears necessary to protect this species.



pH Values

% Survival After 96 Hours

Figure 3



pH Values

% Survival After 96 Hours

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Arcynopteryx parallela

LONG TERM TEMPERATURE TOLERANCE

Date semi-weekly	Days of exposure	Aquarium							
		Control		2		3		4	
		Temp ° C	Number alive	Temp ° C	Number alive	Temp ° C	Number alive	Temp ° C	Number alive
1-09-71	0	13.0	20	14.5	20	18.0	20	19.5	20
1-12-71	3	13.5	20	14.5	20	18.0	9	20.0	2
1-15-71	6	12.5	20	14.5	20	17.5	6	18.5	2
1-19-71	10	13.5	20	15.0	20	18.0	6	19.0	2
1-22-71	13	13.0	20	15.0	20	18.0	6	18.5	2
1-26-71	17	13.0	20	15.0	20	18.0	5	19.0	2
1-29-71	20	13.0	20	15.0	18	18.0	5	19.0	2
2-02-71	24	13.0	20	15.0	15	17.5	2	18.0	2
2-05-71	27	13.0	20	15.5	15	17.5	2	18.5	1
2-09-71	31	13.0	20	14.5	15	17.5	2	20.0	0
2-12-71	34	13.0	20	14.5	14	17.5	2		
2-16-71	38	13.0	20	15.0	12	17.5	2		
2-19-71	41	13.0	20	15.0	11	17.5	2		
2-23-71	45	13.0	20	15.0	8	18.0	2		
2-26-71	48	13.0	20	15.0	5	18.0	1		
3-02-71	52	13.0	20	15.0	5	18.0	1		
3-05-71	55	13.0	20	15.0	5	18.0	1		
3-09-71	59	13.0	20	15.0	3	18.0	0		
3-12-71	62	13.0	20	15.0	0				

Arcynopteryx signata

LONG TERM TEMPERATURE TOLERANCE

Date semi-weekly	Days of exposure	Control		Aquarium 2		3		4	
		Temp ° C	Number alive	Temp ° C	Number alive	Temp ° C	Number alive	Temp ° C	Number alive
4-20-71	0	12.5	10	14.5	10	18.0	10	19.0	10
4-23-71	3	12.5	10	15.0	8	18.0	6	18.0	7
4-27-71	7	12.0	10	15.0	6	18.0	4	18.0	3
4-30-71	10	12.0	10	15.0	6	18.0	4	19.0	3
5-04-71	14	11.5	10	14.0	6	18.0	4	20.0	0
5-07-71	17	12.0	10	15.0	1	18.0	4		
5-11-71	21	12.0	10	15.0	1	18.0	1		
5-14-71	24	13.0	10	15.0	1	18.0	0		
5-18-71	28	12.0	10	14.0	1				
5-21-71	31	13.0	10	14.0	0				

Pteronarcella badia

LONG TERM TEMPERATURE TOLERANCE

Date semi-weekly	Days of exposure	Aquarium Control		2		3		4	
		Temp ° C	Number alive	Temp ° C	Number alive	Temp ° C	Number alive	Temp ° C	Number alive
1-09-71	0	13.0	20	14.5	20	18.0	20	19.5	20
1-12-71	3	12.5	20	14.5	20	18.0	19	20.0	20
1-15-71	6	12.5	20	14.5	20	17.5	19	18.5	20
1-19-71	10	13.5	20	15.0	20	18.0	17	19.0	20
1-22-71	13	13.0	20	15.0	18	18.0	17	18.5	16
1-26-71	17	13.0	20	15.0	18	18.0	17	19.0	15
1-29-71	20	13.0	20	15.0	18	18.0	17	18.5	12
2-02-71	24	13.0	20	15.0	18	17.5	17	18.0	9
2-05-71	27	13.0	20	15.5	16	17.5	15	18.5	6
2-09-71	31	13.0	20	14.5	16	17.5	12	18.5	6
2-12-71	34	12.0	20	14.5	16	17.5	11	19.0	5
2-16-71	38	13.0	20	15.0	16	17.5	9	18.4	3
2-19-71	41	13.0	20	15.0	16	17.5	7	18.5	2
2-23-71	45	13.0	20	15.0	12	18.0	7	19.0	2
2-26-71	48	13.0	20	15.0	10	18.0	4	20.0	2
3-02-71	52	13.0	20	15.0	9	18.0	4	18.5	1
3-05-71	55	13.0	20	15.0	7	18.0	1	19.0	0
3-09-71	59	13.0	20	15.0	7	18.0	1		
3-12-71	62	13.0	20	14.5	5	18.0	1		
3-16-71	66	13.0	20	15.0	5	18.0	1		
3-19-71	69	12.0	20	15.0	5	18.0	1		
3-23-71	73	13.0	20	15.0	4	18.0	1		
3-26-71	76	13.0	20	15.0	4	18.0	0		
3-30-71	80	13.0	20	15.0	4				
4-02-71	83	13.0	20	15.0	1				
4-06-71	87	13.0	20	15.0	0				

Isoperla fulva

LONG TERM TEMPERATURE TOLERANCE

Date semi-weekly	Days of exposure	Control		Aquarium 2		Aquarium 3		Aquarium 4	
		Temp ° C	Number alive	Temp ° C	Number alive	Temp ° C	Number alive	Temp ° C	Number alive
1-15-71	0	12.5	20	14.5	20	17.5	20	18.5	20
1-19-71	4	13.5	20	15.0	9	18.0	9	19.0	3
1-22-71	7	13.0	20	15.0	8	18.0	8	18.5	3
1-26-71	11	13.0	20	15.0	8	18.0	8	19.0	2
1-29-71	14	13.0	20	15.0	2	18.0	5	19.0	2
2-02-71	18	13.0	20	15.0	2	17.5	5	17.0	2
2-05-71	21	13.0	20	15.5	2	17.5	5	18.5	1
2-09-71	25	13.0	20	14.5	1	17.5	5	18.5	1
2-12-71	28	12.0	20	14.5	1	17.5	2	19.0	0
2-16-71	32	13.0	20	15.0	1	17.5	1		
2-19-71	35	13.0	20	15.0	1	17.5	1		
2-23-71	39	13.0	20	15.0	1	18.0	0		
2-26-71	42	13.0	20	15.0	1				
3-02-71	46	13.0	20	15.0	0				

Date semi-weekly	Days of exposure	Aquarium 5	
		Temp ° C	Number alive
1-15-71	0	24.0	20
1-19-71	4	24.5	2
1-22-71	7	24.0	0

Ephemerella grandis

LONG TERM TEMPERATURE TOLERANCE

Date semi-weekly	Days of exposure	Aquarium									
		Control		2		3		4		5	
		Temp ° C	No. alive	Temp ° C	No. alive	Temp ° C	No. alive	Temp ° C	No. alive	Temp ° C	No. alive
1-29-71	0	13.0	10	15.0	10	18.0	10	19.0	10	24.0	10
2-02-71	4	13.0	10	15.0	10	17.5	7	17.0	7	24.0	7
2-05-71	7	13.0	10	15.5	10	17.5	6	18.5	6	24.0	6
2-09-71	11	13.0	10	14.5	10	17.5	6	18.5	6	24.0	2
2-12-71	14	13.0	10	14.5	10	17.5	6	19.0	6	24.0	0
2-16-71	18	13.0	10	15.0	10	17.5	5	18.5	0		
2-19-71	21	13.0	10	15.0	10	17.5	3				
2-23-71	25	13.0	10	15.0	7	18.0	3				
2-26-71	28	13.0	10	15.0	5	18.0	1				
3-02-71	32	13.0	10	15.0	2	18.0	1				
3-05-71	35	13.0	10	15.0	1	18.0	1				
3-09-71	39	13.0	10	15.0	0	18.0	0				

Date semi-weekly	Days of exposure	Aquarium							
		6		7		8		9	
		Temp ° C	No. alive	Temp ° C	No. alive	Temp ° C	No. alive	Temp ° C	No. alive
1-29-71	0	26.0	10	28.0	10	29.0	10	30.0	10
2-02-71	4	26.0	2	27.5	0	29.0	0	30.0	0
2-05-71	7	26.0	0						

Brachycentrus occidentalis

LONG TERM TEMPERATURE TOLERANCE

Date semi-weekly	Days of exposure	Aquarium									
		Control		2		3		4		5	
		Temp ° C	No. alive	Temp ° C	No. alive	Temp ° C	No. alive	Temp ° C	No. alive	Temp ° C	No. alive
1-05-71	0	13.0	20	18.0	20	26.0	20	27.0	20	28.5	20
1-08-71	3	13.0	20	18.0	20	26.0	20	28.0	18	29.0	2
1-12-71	7	13.5	20	18.0	20	26.0	19	28.0	17	29.0	2
1-15-71	10	13.0	20	17.5	19	25.5	16	28.0	11	28.5	1
1-19-71	14	13.5	20	18.0	14	26.0	15	28.0	9	28.5	1
1-22-71	17	13.0	20	18.0	14	26.0	14	28.0	4	29.0	1
1-26-71	21	13.0	20	18.0	14	26.0	14	28.0	3	29.0	0
1-29-71	24	13.0	20	18.0	14	26.0	8	28.0	2	29.0	0
2-02-71	28	13.0	20	17.5	14	26.0	8	27.5	2		
2-05-71	31	13.0	20	17.5	11	26.0	6	27.0	2		
2-09-71	35	13.0	20	17.5	9	24.0	6	27.0	2		
2-12-71	38	13.0	20	17.5	7	26.0	6	27.5	2		
2-16-71	42	13.0	20	17.5	5	26.0	5	27.5	2		
2-19-71	45	13.0	20	17.5	5	26.0	4	27.5	2		
2-23-71	49	13.0	20	18.0	5	25.0	4	27.0	2		
2-26-71	52	13.0	20	18.0	4	25.0	2	27.0	2		
3-02-71	56	13.0	20	18.0	3	25.0	2	28.0	2		
3-05-71	59	13.0	20	18.0	3	26.0	2	27.0	1		
3-09-71	63	13.0	20	18.0	3	26.0	2	27.5	1		
3-12-71	66	13.0	20	18.0	3	26.0	2	26.5	1		
3-16-71	70	13.0	20			25.0	2	27.0	1		
3-19-71	73	13.0	20			26.0	2	27.5	1		
3-23-71	77	13.0	20			26.5	1	28.0	0		
3-26-71	80	13.0	20			25.5	1				
3-30-71	84	13.0	20			25.5	1				
4-02-71	87	13.0	20			25.5	0				

Rhyacophila fuscula

LONG TERM TEMPERATURE TOLERANCE

Date semi-weekly	Days of exposure	Control		Aquarium 2		3		4	
		Temp ° C	Number alive	Temp ° C	Number alive	Temp ° C	Number alive	Temp ° C	Number alive
2-19-71	0	13.0	10	15.0	10	18.0	10	19.0	10
2-23-71	4	13.0	10	15.0	10	18.0	10	19.0	10
2-26-71	7	13.0	10	15.0	10	18.0	10	20.0	9
3-02-71	11	13.0	10	15.0	9	18.0	9	18.5	9
3-05-71	14	13.0	10	15.0	9	18.0	9	20.0	9
3-09-71	18	13.0	10	15.0	9	18.0	9	19.0	9
3-12-71	21	13.5	10	14.5	7	18.0	9	19.0	9
3-16-71	25	12.0	10	15.0	7	18.0	9	18.5	8
3-19-71	28	12.0	10	15.0	7	18.0	8	18.0	8
3-23-71	32	13.0	10	15.0	7	18.0	7	19.0	5
3-26-71	35	13.0	10	14.5	4	18.0	7	19.0	4
3-30-71	39	13.0	10	15.0	4	18.0	7	19.5	3
4-02-71	42	13.0	10	15.0	2	18.0	7	19.5	2
4-06-71	46	13.0	10	15.0	1	18.0	3	20.0	1
4-09-71	49	13.0	10	15.0	1	18.0	3	20.0	1
4-13-71	53	13.5	10	15.0	1	18.0	0	19.0	1
4-16-71	56	12.5	10	15.0	1			19.0	0
4-20-71	60	12.5	10	14.5	1				
4-23-71	63	13.0	10	15.0	0				

Date semi-weekly	Days of exposure	Aquarium 5		6		7	
2-19-71	0	23.0	10	25.0	10	27.0	10
2-23-71	4	23.0	7	25.0	0	27.0	0
2-26-71	7	23.5	6				
3-02-71	11	24.0	3				
3-05-71	14	24.0	1				
3-09-71	18	24.0	0				

Arctopsyche grandis

LONG TERM TEMPERATURE TOLERANCE

Date semi-weekly	Days of exposure	Aquarium Control		2		3		4		5	
		Temp ° C	No. alive	Temp ° C	No. alive	Temp ° C	No. alive	Temp ° C	No. alive	Temp ° C	No. alive
1-04-71	0	13.0	20	14.5	20	18.0	20	19.3	20	25.0	20
1-08-71	4	13.0	20	15.0	18	18.0	20	19.0	17	24.0	2
1-12-71	8	13.5	20	14.5	18	18.0	20	20.0	8	24.0	2
1-15-71	11	12.5	20	14.5	18	17.5	20	18.5	6	24.0	2
1-19-71	15	13.5	20	15.0	18	18.0	18	19.0	4	24.5	1
1-22-71	18	13.0	20	15.0	18	18.0	15	18.5	3	24.0	1
1-26-71	23	13.0	20	15.0	18	18.0	10	19.0	2	23.5	1
1-29-71	26	13.0	20	15.0	17	18.0	9	18.5	2	24.0	1
2-02-71	30	13.0	20	15.0	13	17.5	7	18.0	2	24.0	0
2-05-71	33	13.0	20	15.5	11	17.5	4	18.5	1		
2-09-71	37	13.0	20	14.5	10	17.5	2	18.5	1		
2-12-71	40	12.0	20	14.5	9	17.5	2	20.0	0		
2-16-71	44	13.0	20	15.0	8	17.5	1				
2-19-71	47	13.0	20	15.0	8	17.5	1				
2-23-71	51	13.0	20	15.0	7	18.0	1				
2-26-71	54	13.0	20	15.0	7	18.0	1				
3-02-71	59	13.0	20	15.0	7	18.0	1				
3-05-71	62	13.0	20	15.0	5	18.0	1				
3-09-71	66	13.0	20	15.0	3	18.0	1				
3-12-71	69	13.0	20	14.5	3	18.0	0				
3-16-71	73	12.0	20	15.0	3						
3-19-71	76	12.0	20	15.0	3						
3-23-71	80	13.0	20	15.0	3						
3-26-71	83	13.0	20	15.0	3						
3-30-71	87	13.0	20	15.0	0						

Argia vivida

LONG TERM TEMPERATURE TOLERANCE

Date semi-weekly	Days of exposure	Control		Aquarium 2		3		4	
		Temp ° C	Number alive	Temp ° C	Number alive	Temp ° C	Number alive	Temp ° C	Number alive
3-25-71	0	13.0	10	15.0	10	18.0	10	20.0	10
3-26-71	1	12.5	10	14.5	9	18.0	10	19.0	10
3-30-71	5	13.0	10	15.0	8	18.0	10	19.5	8
4-02-71	8	13.0	10	15.0	5	18.0	10	20.0	8
4-06-71	12	13.0	10	15.0	5	18.0	10	20.0	6
4-09-71	15	13.0	10	15.0	4	18.0	10	20.0	6
4-13-71	19	13.5	10	15.0	4	18.0	10	19.0	6
4-16-71	22	12.5	10	15.0	2	18.0	10	18.0	6
4-20-71	26	12.5	10	14.5	2	18.0	10	19.0	5
4-23-71	29	12.5	10	15.0	1	18.0	5	18.0	2
4-27-71	33	12.0	10	15.0	1	18.0	5	18.0	1
4-30-71	36	12.0	10	15.0	1	18.0	3	19.5	0
5-04-71	40	11.5	10	15.0	1	18.0	2		
5-07-71	43	12.0	10	15.0	1	18.0	1		
5-11-71	47	12.0	10	15.0	0	18.0	1		
5-14-71	50	13.0	10			18.0	0		

Date semi-weekly	Days of exposure	Aquarium 5		6	
		Temp ° C	Number alive	Temp ° C	Number alive
3-25-71	0	24.0	10	26.0	10
3-26-71	1	24.0	10	25.5	8
3-30-71	5	24.0	10	25.5	8
4-02-71	8	24.0	10	25.5	8
4-06-71	12	24.0	8	25.5	8
4-09-71	15	24.0	8	25.5	7
4-13-71	19	24.0	5	25.5	3
4-16-71	22	24.0	4	25.0	2
4-20-71	26	24.0	4	25.0	2
4-23-71	29	24.0	2	25.0	1
4-27-71	33	23.5	1	25.0	1
4-30-71	36	24.0	0	26.0	0

Biblocephala grandis

LONG TERM TEMPERATURE TOLERANCE

Date semi-weekly	Days of exposure	Aquarium: Control		2		3		4	
		Temp ° C	Number alive	Temp ° C	Number alive	Temp ° C	Number alive	Temp ° C	Number alive
1-19-71	0	13.5	10	15.0	10	18.0	10	19.0	10
1-22-71	3	13.0	10	15.0	6	18.0	0	18.5	4
1-26-71	7	13.0	10	15.0	4			19.0	1
1-29-71	10	13.0	10	15.0	2			15.0	0
2-02-71	14	13.0	10	15.0	0				

Date semi-weekly	Days of exposure	Aquarium: 5		6		7		8	
		Temp ° C	Number alive	Temp ° C	Number alive	Temp ° C	Number alive	Temp ° C	Number alive
1-19-71	0	24.0	10	26.0	10	28.0	10	28.5	10
1-22-71	3	24.0	0	26.0	0	28.0	0	29.0	0

Holorusia grandis

LONG TERM TEMPERATURE TOLERANCE

Date semi-weekly	Days of exposure	Aquarium							
		Control		2		3		4	
		Temp ° C	Number alive	Temp ° C	Number alive	Temp ° C	Number alive	Temp ° C	Number alive
1-09-71	0	13.0	20	24.0	20	26.0	20	28.0	20
1-12-71	3	13.5	20	24.0	20	26.0	18	28.0	0
1-15-71	6	12.5	20	24.0	20	26.0	18		
1-19-71	10	13.5	20	24.5	17	26.0	14		
1-22-71	13	13.0	20	24.0	15	26.0	13		
1-26-71	17	13.0	20	23.5	15	26.0	11		
1-29-71	20	13.0	20	24.0	13	26.0	9		
2-02-71	24	13.0	20	24.0	12	26.0	6		
2-05-71	27	13.0	20	24.0	9	26.0	3		
2-09-71	31	13.0	20	24.0	9	25.0	2		
2-12-71	34	13.0	20	24.0	8	26.0	1		
2-16-71	38	13.0	20	24.0	8	26.0	1		
2-19-71	41	13.0	20	24.0	6	26.0	0		
2-23-71	45	13.0	20	23.0	6				
2-26-71	48	13.0	20	23.5	5				
3-02-71	52	13.0	20	25.0	4				
3-05-71	55	13.0	20	24.0	3				
3-09-71	59	13.0	20	24.0	2				
3-12-71	62	12.5	20	24.0	2				
3-16-71	66	12.0	20	24.0	1				
3-19-71	69	12.0	20	24.5	1				
3-23-71	73	13.0	20	25.0	0				

Appendices

Chemical Characteristics

Montana Biological Station

Spring Water

September 15, 1969	PPM
Aluminum	Trace
Barium	3.0
Ca Bicarbonate	135.0
Ca Carbonate	0.0
Carbon Dioxide	1-2
Chlorides	1.5
Chromium	.037
Copper	.02
Hardness (Total)	135
Hydrogen Sulfide	0.0
Fe (Ferric)	.09
Fe (Total)	.12
Fe (Ferrous)	.03
Manganese	.05
Nit. (Ammonia)	.21
Nit. (Nitrate)	Trace
Nit. (Nitrite)	0.0
Oxygen (Dissolved)	8.0
pH Value	7.8
Phenol	None
Phosphate	.11
Silica	6.0
Sulphate	6.0
Temp. Water	6.4° C
Turbidity	4

REACTION OF PTERONARCYS CALIFORNICA TO LOW OXYGEN CONCENTRATIONS

Date initiated - Dec. 1,1970 Flow - 1000 cc/min Temp. - 10° C

Compartment	D.O. Concentration	Days of Survival 4 days	12	26	49	58	97	111
1	2.0	60%	40	40	0	0	0	0
3	2.8	100	100	80	60	20	0	0
5	3.6	100	100	80	60	40	20	20
7	4.0	80	80	80	80	80	20	20
9	4.8	100	100	100	100	100	40	40
14	6.4	100	100	100	100	100	80	0

REACTION OF EPHEMERELLA GRANDIS TO LOW OXYGEN CONCENTRATIONS

Date initiated - Dec. 2,1970 Flow - 1000 cc/min Temp. - 10° C

Compartment	D.O. Concentration	Days of Survival 4 days	12	25	30
2	2.4	10%	0	0	0
4	3.0	50	20	20	0
6	3.6	70	60	20	0
8	4.6	100	70	60	0
10	5.0	80	60	50	0
13	6.0	90	90	80	20

REACTION OF BRACHYCENTRUS OCCIDENTALIS TO LOW OXYGEN CONCENTRATIONS

Date initiated - Oct. 16,1970 Flow - 500 cc/min Temp. - 10° C

Compartment	D.O. Concentration	Days of Survival 4 days	9	30	38	44	52	56	70	92	102	120
2	2.0	90%	90	90	90	90	90	90	90	90	70	70
4	3.2	90	90	90	90	90	90	90	90	80	80	50
6	4.0	90	90	70	70	60	60	60	60	60	60	50
8	4.8	100	100	100	100	100	90	90	90	90	70	70
10	5.2	100	100	100	100	100	100	100	90	90	90	80
12	6.4	100	100	100	100	100	100	100	100	90	90	80

REACTION OF ACRONEURIA PACIFICA TO LOW OXYGEN CONCENTRATIONS

Date Initiated - Dec. 1,1969 Flow - 1000 cc/min Temp. - 10° C

Compartment	D.O. Concentration	Days of Survival 4 days	12	26	49	58	77	99	111
1	1.6	100%	90	70	20	10	10	10	10
3	3.2	100	100	90	90	90	60	40	30
5	4.4	100	100	100	70	70	60	30	30
7	5.8	100	100	100	80	80	70	60	50
9	6.4	100	100	100	50	50	50	30	30
14	8.4	100	100	100	90	90	90	70	50

REACTION OF ATHERIX VARIEGATA TO LOW OXYGEN CONCENTRATIONS

Date Initiated - Dec. 2,1969 Flow - 1000 cc/min Temp. - 10° C

Compartment	D.O. Concentration	Days of Survival 4 days	15	30	40
2	2.4	90%	90	90	90
4	4.0	100	100	100	100
6	5.6	100	100	100	100
8	6.0	100	100	100	100
10	6.8	100	100	100	100
13	8.0	100	100	100	100

REACTION OF RHYACOPHILA FUSCULA TO LOW OXYGEN CONCENTRATIONS

Date Initiated - Jan. 12,1970 Flow - 1000 cc/min Temp. - 10° C

Compartment	D.O. Concentration	Days of Survival 4 days	8
2	2.4	20%	20
4	4.0	20	20
6	5.6	60	0
8	6.0	80	20
10	6.8	100	40
13	8.0	100	50

REACTION OF HYDROPSYCHE SP. TO LOW OXYGEN CONCENTRATIONS

Date Initiated - Dec. 1,1969 Flow - 1000 cc/min Temp. - 10° C

Compartment	D.O. Concentration	Days of Survival 4 days	12	26	49	50
1	1.6	0%	0	0	0	0
3	2.4	0	0	0	0	0
5	3.2	0	0	0	0	0
7	4.0	90	70	40	20	20
9	4.8	80	70	60	30	30
14	8.0	90	90	90	90	50

REACTION OF ARCYNOPTERYX AUREA TO LOW OXYGEN CONCENTRATIONS

Date Initiated - Dec. 8,1970 Flow - 1000 cc/min Temp. - 10° C

Compartment	D.O. Concentration	Days of Survival 4 days	12	26
2	2.0	10%	0	0
4	2.8	20	10	0
6	3.6	70	40	0
8	4.4	40	20	10
10	4.8	70	30	0
13	5.6	100	90	0

REACTION OF PTERONARCELLA BADIA TO LOW OXYGEN CONCENTRATIONS

Date Initiated - Dec. 12,1970 Flow - 1000 cc/min Temp. - 10° C

Compartment	D.O. Concentration	Days of Survival 4 days	16	35	57	69
2	2.0	60%	20	0	0	0
4	2.8	60	50	30	10	10
6	3.6	60	60	50	30	30
8	4.4	90	90	90	90	50
10	4.8	50	20	20	20	20
13	5.6	100	100	90	80	40

REACTION OF NEMOURA CINCTIPES TO LOW OXYGEN CONCENTRATIONS

Date initiated - Dec. 19,1970 Flow - 1000 cc/min Temp. - 10° C

Compartment	D.O. Concentration	Days of Survival 4 days	8
1	1.6	0%	0
3	2.4	20	0
5	3.2	40	0
7	4.0	80	10
9	4.8	100	20
14	8.0	100	40

REACTION OF ARCYNOPTERYX PARALLELA TO LOW OXYGEN CONCENTRATIONS

Date initiated - Feb. 17,1970 Flow - 1000 cc/min Temp. - 10° C

Compartment	D.O. Concentration	Days of Survival 4 days	22	34
1	1.6	60%	0	0
3	2.4	100	50	0
5	3.2	100	60	0
7	4.0	100	80	10
9	4.8	100	80	10
14	8.0	80	80	10

REACTION OF GAMMARUS LACUSTRIS SARS TO SULFURIC ACID

RESPONSE	pH 2.0	pH 3.0	pH 4.0	pH 5.0	pH 6.0
24 Hours					
% Unaffected	0	0	0	50	80
% Affected	0	0	25	25	10
% Dead	100	100	75	25	10
48 Hours					
% Unaffected	0	0	0	25	55
% Affected	0	0	0	15	5
% Dead	100	100	100	60	40
72 Hours					
% Unaffected	0	0	0	15	55
% Affected	0	0	0	5	5
% Dead	100	100	100	80	40
96 Hours					
% Unaffected	0	0	0	15	55
% Affected	0	0	0	5	5
% Dead	100	100	100	80	40

REACTION OF GAMMARUS LACUSTRIS SARS TO HYDROCHLORIC ACID

RESPONSE	pH 2.0	pH 3.0	pH 4.0	pH 5.0	pH 6.0
24 Hours					
% Unaffected	0	0	0	75	80
% Affected	0	0	45	10	20
% Dead	100	100	55	15	0
48 Hours					
% Unaffected	0	0	0	55	90
% Affected	0	0	25	5	0
% Dead	100	100	75	40	10
72 Hours					
% Unaffected	0	0	0	30	75
% Affected	0	0	5	0	0
% Dead	100	100	95	70	25
96 Hours					
% Unaffected	0	0	0	20	75
% Affected	0	0	0	0	0
% Dead	100	100	100	80	25

REACTION OF HOLORUSIA SPP. TO SULFURIC ACID

RESPONSE	pH 2.0	pH 3.0	pH 4.0	pH 5.0	pH 6.0
24 Hours					
% Unaffected	45	70	95	100	100
% Affected	20	30	5	0	0
% Dead	35	0	0	0	0
48 Hours					
% Unaffected	0	70	95	100	100
% Affected	15	20	5	0	0
% Dead	85	10	0	0	0
72 Hours					
% Unaffected	0	70	90	85	100
% Affected	0	15	5	5	0
% Dead	100	15	5	10	0
96 Hours					
% Unaffected	0	60	80	85	100
% Affected	0	0	5	5	0
% Dead	100	40	15	10	0

REACTION OF HOLORUSIA SPP. TO HYDROCHLORIC ACID

RESPONSE	pH 2.0	pH 3.0	pH 4.0	pH 5.0	pH 6.0
24 Hours					
% Unaffected	25	75	100	100	100
% Affected	15	15	0	0	0
% Dead	60	10	0	0	0
48 Hours					
% Unaffected	5	60	100	95	100
% Affected	5	15	0	0	0
% Dead	90	25	0	5	0
72 Hours					
% Unaffected	0	55	100	95	100
% Affected	0	5	0	0	0
% Dead	100	40	0	5	0
96 Hours					
% Unaffected	0	30	85	95	100
% Affected	0	5	0	0	0
% Dead	100	65	15	5	0

REACTION OF RHITHROGENA ROBUSTA DODDS
TO SULFURIC ACID

RESPONSE	pH 2.0	pH 3.0	pH 4.0	pH 5.0	pH 6.0
24 Hours					
% Unaffected	0	0	75	80	100
% Affected	0	35	20	10	0
% Dead	100	65	5	10	0
48 Hours					
% Unaffected	0	0	70	80	100
% Affected	0	0	10	5	0
% Dead	100	100	20	15	0
72 Hours					
% Unaffected	0	0	70	80	100
% Affected	0	0	10	5	0
% Dead	100	100	20	15	0
96 Hours					
% Unaffected	0	0	35	80	100
% Affected	0	0	0	5	0
% Dead	100	100	65	15	0

REACTION OF RHITHROGENA ROBUSTA DODDS
TO HYDROCHLORIC ACID

RESPONSE	pH 2.0	pH 3.0	pH 4.0	pH 5.0	pH 6.0
24 Hours					
% Unaffected	0	0	60	85	100
% Affected	0	5	15	10	0
% Dead	100	95	25	5	0
48 Hours					
% Unaffected	0	0	60	80	95
% Affected	0	0	5	0	0
% Dead	100	100	35	20	5
72 Hours					
% Unaffected	0	0	45	80	95
% Affected	0	0	0	0	0
% Dead	100	100	55	20	5
96 Hours					
% Unaffected	0	0	45	80	95
% Affected	0	0	0	0	0
% Dead	100	100	55	20	5

REACTION OF EPHEMERELLA DODDSI NEEDHAM
TO SULFURIC ACID

RESPONSE	pH 2.0	pH 3.0	pH 4.0	pH 5.0	pH 6.0
24 Hours					
% Unaffected	0	0	85	100	100
% Affected	0	25	15	0	0
% Dead	100	75	0	0	0
48 Hours					
% Unaffected	0	0	70	95	100
% Affected	0	15	15	5	0
% Dead	100	85	15	0	0
72 Hours					
% Unaffected	0	0	60	95	95
% Affected	0	15	5	5	0
% Dead	100	85	35	0	5
96 Hours					
% Unaffected	0	0	60	90	95
% Affected	0	0	5	5	0
% Dead	100	100	35	5	5

REACTION OF EPHEMERELLA DODDSI NEEDHAM
TO HYDROCHLORIC ACID

RESPONSE	pH 2.0	pH 3.0	pH 4.0	pH 5.0	pH 6.0
24 Hours					
% Unaffected	0	0	85	100	100
% Affected	0	0	10	0	0
% Dead	100	100	5	0	0
48 Hours					
% Unaffected	0	0	60	100	100
% Affected	0	0	0	0	0
% Dead	100	100	40	0	0
72 Hours					
% Unaffected	0	0	60	90	100
% Affected	0	0	0	5	0
% Dead	100	100	40	5	0
96 Hours					
% Unaffected	0	0	60	85	100
% Affected	0	0	0	0	0
% Dead	100	100	40	15	0

REACTION OF ARCTOPSYCHE GRANDIS (BANKS)
TO SULFURIC ACID

RESPONSE	pH 2.0	pH 3.0	pH 4.0	pH 5.0	pH 6.0
24 Hours					
% Unaffected	0	40	75	100	100
% Affected	25	50	25	0	0
% Dead	75	10	0	0	0
48 Hours					
% Unaffected	0	40	75	100	100
% Affected	0	40	25	0	0
% Dead	100	20	0	0	0
72 Hours					
% Unaffected	0	20	75	95	95
% Affected	0	30	15	0	0
% Dead	100	50	10	5	5
96 Hours					
% Unaffected	0	15	55	90	95
% Affected	0	25	10	0	0
% Dead	100	60	35	10	5

REACTION OF ARCTOPSYCHE GRANDIS (BANKS)
TO HYDROCHLORIC ACID

RESPONSE	pH 2.0	pH 3.0	pH 4.0	pH 5.0	pH 6.0
24 Hours					
% Unaffected	15	85	100	100	100
% Affected	15	10	0	0	0
% Dead	70	5	0	0	0
48 Hours					
% Unaffected	0	65	85	100	100
% Affected	0	5	0	0	0
% Dead	100	30	15	0	0
72 Hours					
% Unaffected	0	65	85	100	100
% Affected	0	5	0	0	0
% Dead	100	30	15	0	0
96 Hours					
% Unaffected	0	65	85	95	100
% Affected	0	5	0	0	0
% Dead	100	30	15	5	0

REACTION OF ARCYNOPTERYX PARALLELA FRISON TO SULFURIC ACID

RESPONSE	pH 2.0	pH 3.0	pH 4.0	pH 5.0	pH 6.0
24 Hours					
% Unaffected	0	0	55	90	100
% Affected	0	5	25	10	0
% Dead	100	95	20	0	0
48 Hours					
% Unaffected	0	0	50	100	100
% Affected	0	0	20	0	0
% Dead	100	100	30	0	0
72 Hours					
% Unaffected	0	0	45	100	100
% Affected	0	0	25	0	0
% Dead	100	100	30	0	0
96 Hours					
% Unaffected	0	0	25	85	100
% Affected	0	0	20	0	0
% Dead	100	100	55	15	0

REACTION OF ARCYNOPTERYX PARALLELA FRISON TO HYDROCHLORIC ACID

RESPONSE	pH 2.0	pH 3.0	pH 4.0	pH 5.0	pH 6.0
24 Hours					
% Unaffected	0	0	55	100	100
% Affected	0	0	10	0	0
% Dead	100	100	35	0	0
48 Hours					
% Unaffected	0	0	35	95	100
% Affected	0	0	0	0	0
% Dead	100	100	65	5	0
72 Hours					
% Unaffected	0	0	35	95	100
% Affected	0	0	0	0	0
% Dead	100	100	65	5	0
96 Hours					
% Unaffected	0	0	5	75	100
% Affected	0	0	0	0	0
% Dead	100	100	95	25	0

REACTION OF PTERONARCELLA BADIA (HAGEN)
TO SULFURIC ACID

RESPONSE	pH 2.0	pH 3.0	pH 4.0	pH 5.0	pH 6.0
24 Hours					
% Unaffected	0	0	65	95	100
% Affected	0	40	35	5	0
% Dead	100	60	0	0	0
48 Hours					
% Unaffected	0	0	65	95	100
% Affected	0	0	25	5	0
% Dead	100	100	10	0	0
72 Hours					
% Unaffected	0	0	55	95	100
% Affected	0	0	15	5	0
% Dead	100	100	30	0	0
96 Hours					
% Unaffected	0	0	55	90	100
% Affected	0	0	15	5	0
% Dead	100	100	30	5	0

REACTION OF PTERONARCELLA BADIA (HAGEN)
TO HYDROCHLORIC ACID

RESPONSE	pH 2.0	pH 3.0	pH 4.0	pH 5.0	pH 6.0
24 Hours					
% Unaffected	0	10	85	100	100
% Affected	0	10	15	0	0
% Dead	100	80	0	0	0
48 Hours					
% Unaffected	0	0	70	100	100
% Affected	0	0	5	0	0
% Dead	100	100	25	0	0
72 Hours					
% Unaffected	0	0	35	100	100
% Affected	0	0	0	0	0
% Dead	100	100	65	0	0
96 Hours					
% Unaffected	0	0	15	90	100
% Affected	0	0	0	0	0
% Dead	100	100	85	10	0

REACTION OF ISOPERLA FULVA CLAASSEN TO SULFURIC ACID

RESPONSE	pH 2.0	pH 3.0	pH 4.0	pH 5.0	pH 6.0
24 Hours					
% Unaffected	0	0	15	95	100
% Affected	0	0	35	5	0
% Dead	100	100	50	0	0
48 Hours					
% Unaffected	0	0	10	95	100
% Affected	0	0	10	0	0
% Dead	100	100	80	5	0
72 Hours					
% Unaffected	0	0	10	80	100
% Affected	0	0	10	5	0
% Dead	100	100	80	15	0
96 Hours					
% Unaffected	0	0	10	80	100
% Affected	0	0	10	5	0
% Dead	100	100	80	15	0

REACTION OF ISOPERLA FULVA CLAASSEN TO HYDROCHLORIC ACID

RESPONSE	pH 2.0	pH 3.0	pH 4.0	pH 5.0	pH 6.0
24 Hours					
% Unaffected	0	0	15	95	100
% Affected	0	0	10	0	0
% Dead	100	100	75	5	0
48 Hours					
% Unaffected	0	0	15	75	100
% Affected	0	0	5	0	0
% Dead	100	100	80	25	0
72 Hours					
% Unaffected	0	0	0	75	100
% Affected	0	0	0	0	0
% Dead	100	100	100	25	0
96 Hours					
% Unaffected	0	0	0	75	100
% Affected	0	0	0	0	0
% Dead	100	100	100	25	0

REACTION OF ACRONEURIA PACIFICA BANKS
TO SULFURIC ACID

RESPONSE	pH 2.0	pH 3.0	pH 4.0	pH 5.0	pH 6.0
24 Hours					
% Unaffected	0	0	65	85	100
% Affected	0	0	35	15	0
% Dead	100	100	0	0	0
48 Hours					
% Unaffected	0	0	65	85	100
% Affected	0	0	35	15	0
% Dead	100	100	0	0	0
72 Hours					
% Unaffected	0	0	65	85	100
% Affected	0	0	10	10	0
% Dead	100	100	25	5	0
96 Hours					
% Unaffected	0	0	55	85	100
% Affected	0	0	5	10	0
% Dead	100	100	40	5	0

REACTION OF ACRONEURIA PACIFICA BANKS
TO HYDROCHLORIC ACID

RESPONSE	pH 2.0	pH 3.0	pH 4.0	pH 5.0	pH 6.0
24 Hours					
% Unaffected	0	20	90	100	100
% Affected	0	25	10	0	0
% Dead	100	55	0	0	0
48 Hours					
% Unaffected	0	0	90	100	100
% Affected	0	0	10	0	0
% Dead	100	100	0	0	0
72 Hours					
% Unaffected	0	0	85	100	100
% Affected	0	0	0	0	0
% Dead	100	100	15	0	0
96 Hours					
% Unaffected	0	0	85	100	100
% Affected	0	0	0	0	0
% Dead	100	100	15	0	0

REACTION OF EPHEMERELLA GRANDIS GRANDIS EATON TO SULFURIC ACID

RESPONSE	pH 2.0	pH 3.0	pH 4.0	pH 5.0	pH 6.0
24 Hours					
% Unaffected	0	40	65	95	100
% Affected	0	15	25	5	0
% Dead	100	45	10	0	0
48 Hours					
% Unaffected	0	0	60	90	100
% Affected	0	0	15	0	0
% Dead	100	100	26	10	0
72 Hours					
% Unaffected	0	0	60	90	100
% Affected	0	0	15	0	0
% Dead	100	100	25	10	0
96 Hours					
% Unaffected	0	0	60	90	100
% Affected	0	0	15	0	0
% Dead	100	100	25	10	0

REACTION OF EPHEMERELLA GRANDIS GRANDIS EATON TO HYDROCHLORIC ACID

RESPONSE	pH 2.0	pH 3.0	pH 4.0	pH 5.0	pH 6.0
24 Hours					
% Unaffected	0	35	95	100	100
% Affected	0	10	5	0	0
% Dead	100	55	0	0	0
48 Hours					
% Unaffected	0	0	95	85	100
% Affected	0	0	5	0	0
% Dead	100	100	0	15	0
72 Hours					
% Unaffected	0	0	65	85	100
% Affected	0	0	0	0	0
% Dead	100	100	35	15	0
96 Hours					
% Unaffected	0	0	65	85	95
% Affected	0	0	0	0	0
% Dead	100	100	35	15	5